ARCHIVES OF PATHOLOGY

VOLUME 43 FEBRUARY 1947 NUMBER 2

DEVELOPMENT OF BONE MARROW IN ADULT ANIMALS

BERNHARD STEINBERG, M.D.
AND
VIRGINIA HUFFORD, A.B.
TOLEDO, OHIO

THE DEVELOPMENT of cellular elements of bone marrow has been studied in fetal life, in young animals [1] and in cultures of explanted marrow.[2] Investigators have observed the progressive regeneration of marrow cells that were recovering from hypoplasia induced by chemicals,[3] starvation [4] and disease.[5] In this article we present studies of the regeneration of the complete marrow, including stroma, fat spaces, frequently referred to as fat cells, and myeloid cells. The marrow was removed mechanically. Thus the generalized systemic effects incident to previously used methods were avoided.[6]

Bone marrow consists of stromal argyrophil reticulum fibers, reticular cells, fat spaces, blood vessels and myeloid elements composed of erythrocytes, granulocytes and megakaryocytes. Some investigators include monocytes. The role of the primitive reticular cell is involved in the subject of the well known controversy that has arisen among hematologists. The monophyletic school (Ferrata, Pappenheim, Maximow) believes that one primitive cell, variously labeled as hemocytoblast, lymphoidocyte and free undifferentiated reticulum cell, gives rise to all types of blood cells. The polyphyletic school, including the duolists and trialists (Ehrlich, Nägeli, Piney, Sabin) ascribes multiple origins to blood cells. The composite and the more prevailing views of that school are that the myeloblast in the marrow gives rise to granular leukocytes, that the erythrocytes are derived from the endothelium of the vessels of the marrow and that the lymphocytes arise from the lymphoblasts of lymphoid tissue. The views on the origin of monocytes also vary;

From the Toledo Hospital Institute of Medical Research.

1. Sabin, F. R.; Metler, F. R.; Smithburn, K. D.; Thomas, R. M., and Hummel, L. E.: J. Exper. Med. **64**:97, 1936.

2. Osgood, E. E.: J. A. M. A. **109**:933, 1937. Rachmilewitz, M., and Rosin, D.: Am. J. M. Sc. **206**:17, 1943.

3. Muller, G. L.: J. Exper. Med. **43**:533, 1926.

4. Doan, C. A.; Cunningham, R. S., and Sabin, F. R.: Contrib. Embryol. **16**:163, 1925.

5. Peabody, F. W.: Am. J. Path. **2**:487, 1926.

6. Steinberg, B., and Martin, R. A.: Proc. Soc. Exper. Biol. & Med. **61**:428, 1946.

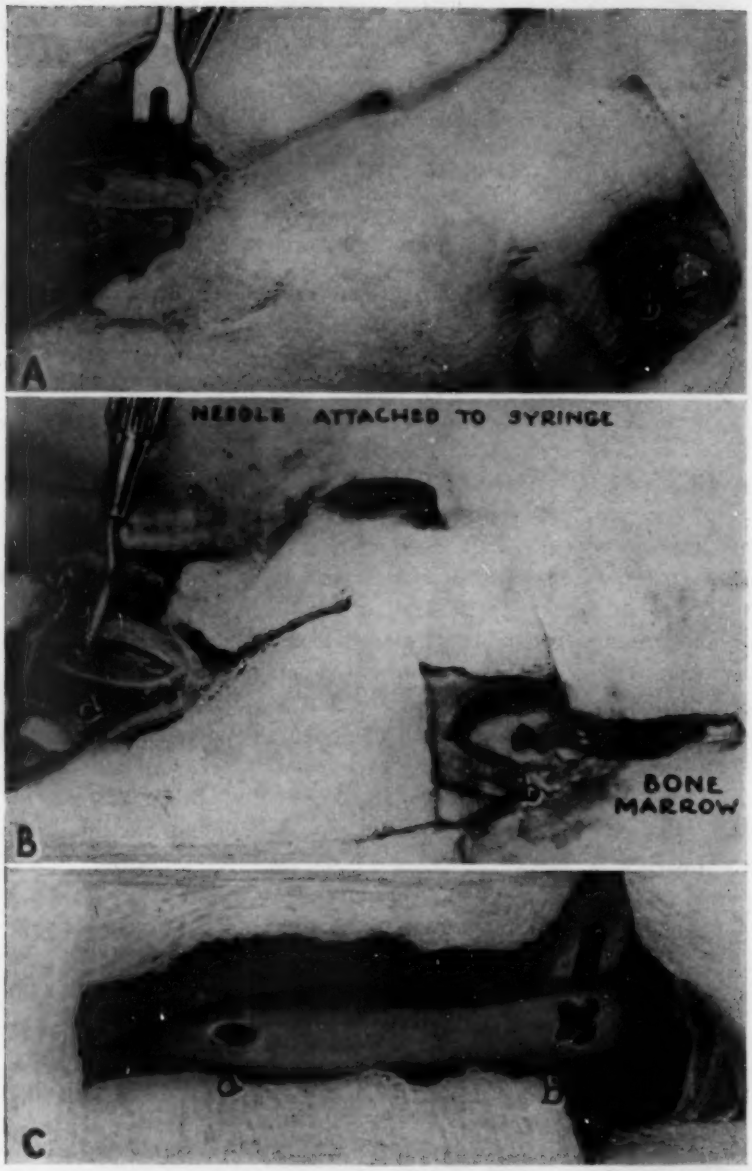


Fig. 1.—Removal of marrow from a long bone of a living rabbit. Holes are drilled in the bone with a Ralk nail drill. At one end of the bone a single hole is made (*a* in *A*). At the opposite end, four holes are made, and the central bone spicule is removed, leaving a large opening (*b* in *A*). A flexible silver cannula is inserted into the single hole (*a* in *B*), and with a syringe containing oil or water the marrow is expressed through the larger opening (*b* in *B*). Both openings are then sealed with bone wax (*a* and *b* in *C*).

the fixed macrophage, the vascular endothelial cell and the lymphoblast are the variously assumed sites of origin. There are many variables of these major theories of hemopoiesis.

The nomenclature of immature cells reflects in part the conflicting views of origin and development. Since the morphologic aspect of a developing cell is necessarily variable, a confusing motley of terms has come into being. For the purpose of this paper the following terms will be employed: "primitive reticular cell," which includes the intermediate forms; occasionally the term "hemocytoblast," used in the sense of an intermediate form of the primitive reticular cell; "myeloblasts"; "myelocytes"; "juveniles"; "stabs"; "mature polymorphonuclear

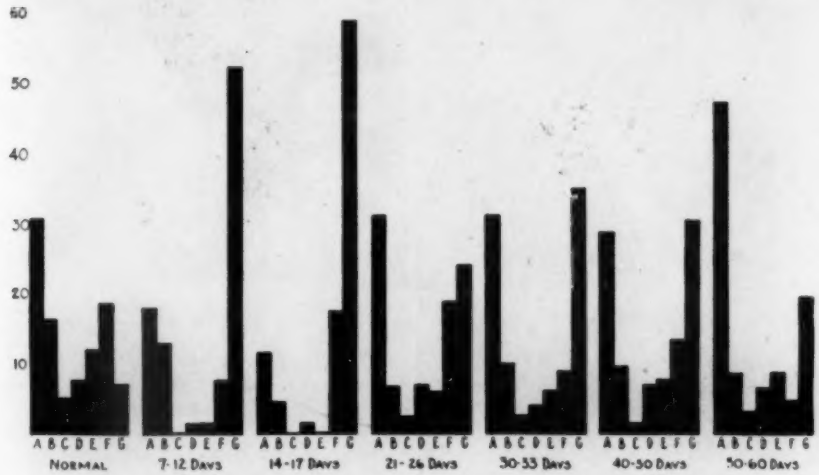


Fig. 2.—Distribution of myeloid cells of various forms in regenerating marrow at various periods following extirpation. *A*, nucleated red blood cells; *B*, polymorphonuclear cells; *C*, stabs; *D*, juveniles; *E*, myelocytes; *F*, myeloblasts; *G*, primitive cells.

cells." All the progenitors of the erythrocytes will be grouped as nucleated red blood cells, and the platelet precursors, as megakaryocytes and megakaryoblasts.

The primitive reticular cell is elongated and contains an ovoid nucleus with a fairly distinct perinuclear membrane and little chromatin. There may be one or two nucleoli. The cytoplasm is faintly basophilic, homogeneous and frequently not apparent. There are intermediate forms with round nuclei and with a greater amount of basophilic cytoplasm. As the primitive reticular cell assumes a more or less round shape, the chromatin increases in amount, the nucleus becomes round and occasionally indented, the cytoplasm becomes more basophilic and shows vacuoles. This cell is probably the hemocytoblast of Ferrata and of Maximow, Nägeli's myeloblast, Pappenheim's lymphoidocyte and the

lymphoid hemoblast of Jordan. The primitive reticular cell is seldom transformed to the hemocytoblast in the marrow normally. Under normal conditions hemopoiesis is homoplastic. Mature cells are derived from the younger forms. In pathologic states and under certain experimental procedures there is heteroplastic hemopoiesis, in which myelo-

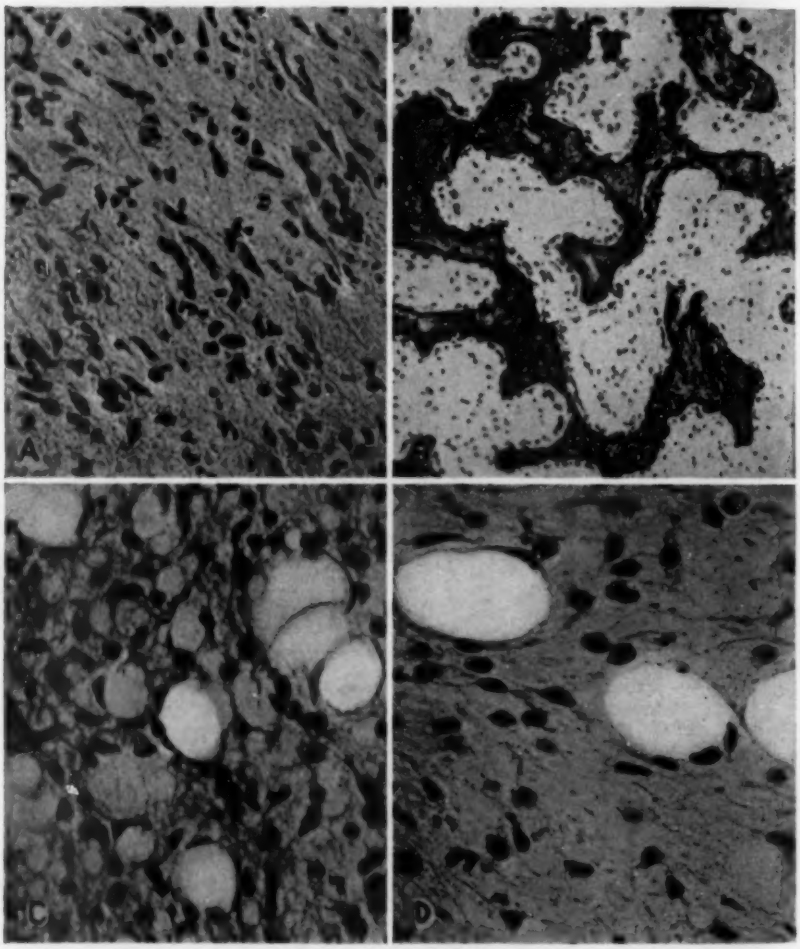


Fig. 3.—*A*, sheets of primitive reticular cells originating from the tibial endosteum nine days after extirpation of the marrow. Small capillaries are present with polymorphonuclear leukocytes that were derived from the circulation.

B, bone trabeculae extending from the tibial endosteum into the marrow cavity, with sheets of primitive reticular cells arising from the endosteum of the trabeculae.

C, grouping of two or more of the reticular cells. Vacuoles appear in the cytoplasm, and a few fat spaces begin to appear.

D, coalescence of the cytoplasm of reticular cells giving rise to fat spaces. The nuclei of reticular cells have migrated to the peripheries of fat spaces. Many reticular cells persist.

cytes and erythroblasts may be derived from the primitive reticular cell and its intermediate forms.

METHOD

The marrow of one or two of the long bones (tibias in these experiments) of the rabbit was extirpated by a method described previously.[6] An incision was made at each end of a tibia. A single opening was made at the narrow end and four openings at the broad end with a Ralk nail drill. The piece of bone outlined by the four openings was lifted out. A tight fitting flexible silver cannula was inserted into the single opening. A syringe filled with sterile liquid petrolatum was attached to the cannula. The pressure of the oil separated the marrow and expressed it out of the bone cavity through the larger opening (fig. 1). The cavity of the bone was flushed out repeatedly with a warm solution of sodium chloride. In some of the animals the marrow was removed from the epiphyses as well as from the shaft. One or both of the ends were packed with inert material to prevent reformation of marrow.[7] In other rabbits the epiphysial marrow was left intact.

Marrow was extirpated from 60 tibias (the right or both tibias) of 44 rabbits. The ages of the animals ranged from 8 to 10 months. One or more of the animals were killed at intervals of one to sixty days. The contents of the tibias were fixed in formaldehyde and Bouin's solutions. Sections were cut at various levels of the entire length and width of the marrow. The marrow was stained with hematoxylin, eosin and Giemsa preparations. The marrow was studied for the following factors: (1) rapidity and extent of formation of fat spaces and reticulum, (2) completeness with which the marrow refilled the tubular shaft of the tibia, (3) reestablishment of the normal proportions of the cell types and (4) the return to normal of the numerical content of individual and all cell types.

To determine the normal values, the absolute and the relative number of cells of each type were determined in the marrow removed at the onset of the experiment. Whenever only one tibial marrow was extirpated for regeneration, the cell content of the marrow of the other tibia was also determined when the animal was killed. As the animals were put to death, the cells of the regenerated marrow were counted and classified as to types.

RESULTS

Within the first nine days after the marrow was extirpated, the endosteum began to send out offshoots composed of sheets of primitive reticular cells and bone trabeculae (fig. 3 *A*). Extending from the endosteal layer of the trabeculae and bridging them were more sheets of primitive reticular cells (3 *B*). Some of the reticular cells began to change from ovoid to round forms, and vacuoles appeared in the cytoplasm, which became more abundant and basophilic. Two or more of the reticular cells became grouped, the cytoplasmic vacuoles coalesced and a fat space was formed (fig. 3 *C* and *D*). The formation of fat spaces continued for at least two months. Capillaries began to appear within the nine day period (fig. 3 A). Myeloid cells were few and

7. Steinberg, B., and Martin, R. A.: Proc. Soc. Exper. Biol. & Med. **63**:390, 1946.

scattered. Nucleated red blood cells predominated. Mature polymorphonuclear leukocytes were within capillaries and were derived from the circulation.

Between twelve and seventeen days after extirpation of the marrow, islands of hemocytoblasts, myeloblasts and erythroblasts and an infre-

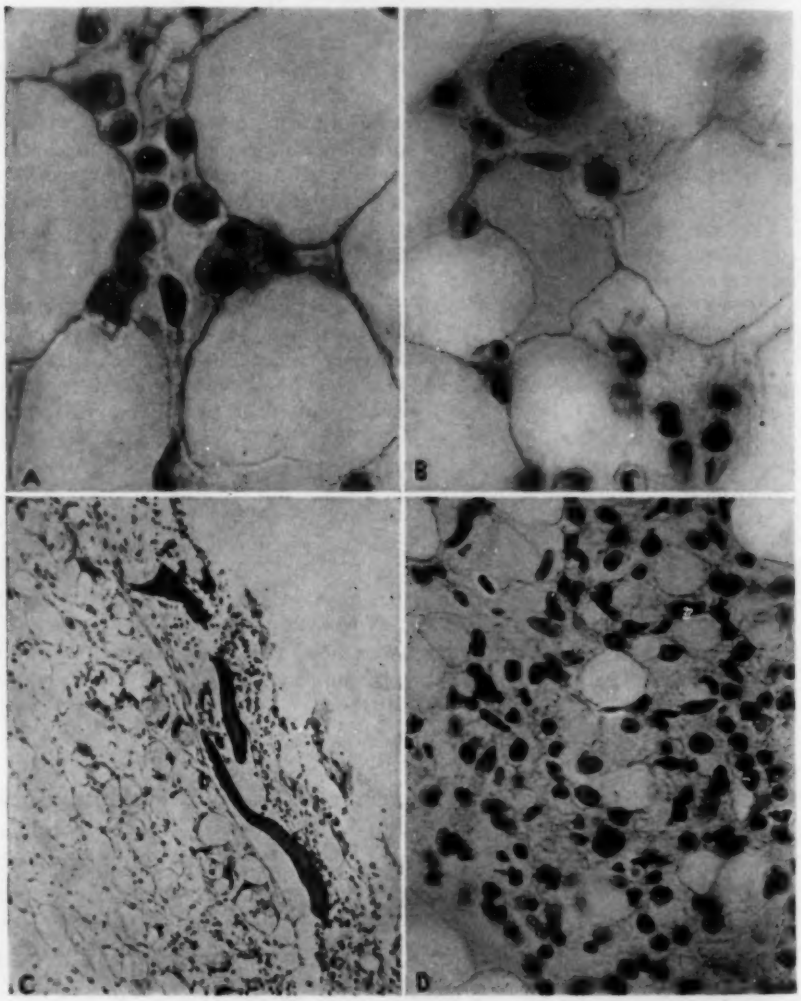


Fig. 4.—*A*, one of the islands of myeloid cells appearing from the twelfth to the seventeenth day after extirpation of marrow. In *B* there is a megakaryoblast.

C and *D*, regeneration twenty-one days after extirpation of marrow. The marrow cavity is almost completely filled, but the development of the marrow is not uniform. Bone trabeculae and small islands of reticular cells persist. The myeloid cell content is still small in numbers.

quent megakaryoblast were seen throughout the reforming marrow (fig. 4 *A* and *B*). The myeloid cells were found to congregate at the margins

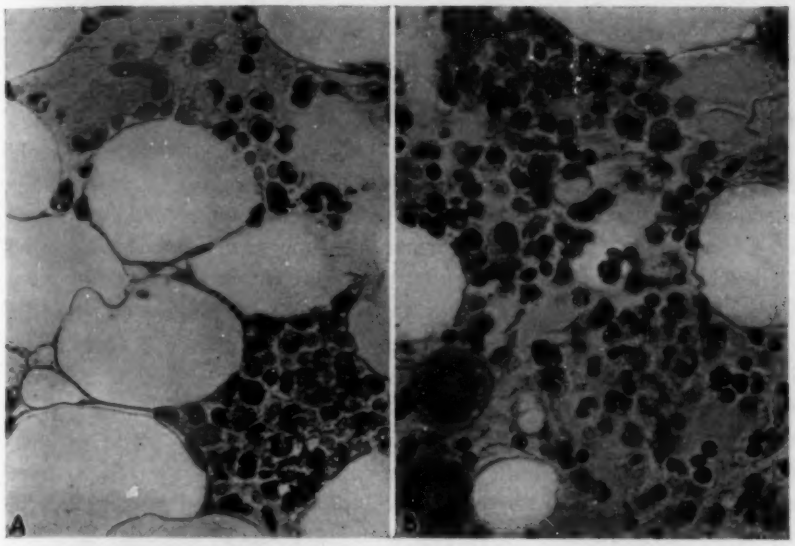


Fig. 5.—*A*, regeneration thirty days after extirpation of marrow. The myeloid areas are of average normal proportions. There is formation of a fat space at the upper left.

B, regeneration forty-three days after extirpation of marrow. Megakaryocytes appear in numbers consistent with normal marrow. There is a total increase in myeloid cells.

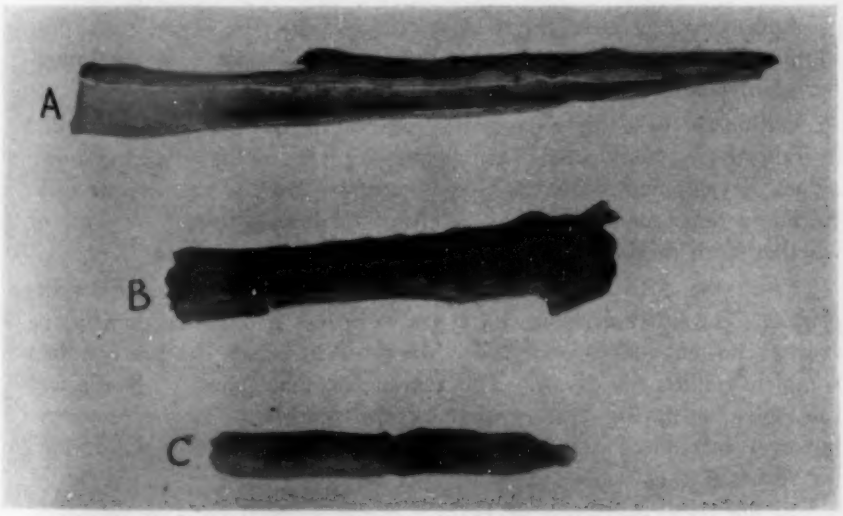


Fig. 6.—*A*, shaft of a bone from which marrow has been completely removed. The animal was killed to determine the efficacy of the procedure of complete removal of marrow. *B*, normal marrow which was removed from the shaft. *C*, regenerated marrow present thirty days after marrow had been completely removed from the shaft of a tibia.

of, or between, fat spaces. Primitive reticular cells predominated (fig. 2). The calcium of the bone trabeculae became reduced or disappeared entirely.

On the twenty-first day after extirpation the greater part of the marrow cavity had become filled. However, there was no uniformity of appearance. In some areas there was slight to moderate myeloid cellularity, with all the cell types represented. In other places the bone trabeculae and small collections of primitive reticular cells persisted (fig. 4 *C* and *D*). The relative formation of marrow elements suggested that development of fat spaces was prerequisite for formation of myeloid cells. Nucleated red blood cells and myeloblasts predominated (fig. 2).

The disappearance of bone trabeculae began with the absorption of calcium. The endosteal layer with its osteoblasts migrated away from the trabeculae and became a part of reticular cell sheets. The fate of the osteocytes was difficult to evaluate. Some of them showed gradual stages of disintegration and final absorption; others appeared to have become integrated in the myeloid development. The disappearance of bone trabeculae was by no means uniform. They persisted in some animals for sixty days after extirpation of marrow.

In thirty days the number of myeloid cells increased appreciably (fig. 2). Erythroblasts began to appear in larger numbers. Whereas in some areas the marrow was within the average normal in numbers and distribution of myeloid cell types, in other parts the picture was not unlike that of nine to seventeen days after extirpation (fig. 5 *A*).

Approximately after thirty days, megakaryocytes began to appear in numbers consistent with average normal distribution. The total number of myeloid cells became increased (fig. 5 *B*).

Within the first thirty days after extirpation the observations tended to indicate that homoplastic hemopoiesis represened the sole mechanism of cell development. In subsequent periods there were obvious difficulties in determining whether heteroplastic hemopoiesis took over or whether both mechanisms continued to operate.

From the fiftieth day the erythroblastic elements became hyperplastic. The other myeloid cell types were less in number than in the average normal marrow (fig. 2). In most instances the marrow had completely filled the cavity. The average normal quantity of cells was reestablished in the greater part of the marrow. There remained, however, infrequent islands of primitive reticular cells and partly formed fat spaces.

In the bones with one or both of the epiphysial ends sealed off to prevent reformation of marrow, regeneration in the shafts was decreased appreciably. The primitive reticular cells persisted in greater numbers for longer periods. The total myeloid content remained appreciably

lower even at the end of two months. Eventually the entire shaft became filled with marrow.

Comparison of the regenerating marrow of the adult rabbit with that of the fetus and the young animal suggests a comparable development (fig. 6).

SUMMARY

The complete marrow of one or both tibias was extirpated in 44 living rabbits. The marrow was allowed to regenerate. Animals were killed at intervals for a period of two months, and regeneration was studied. The probable development of marrow is indicated within the limits of a morphologic experiment.

The reformation of marrow began from the tibial endosteum with offshoots of primitive reticular cells and formation of bone spicules.

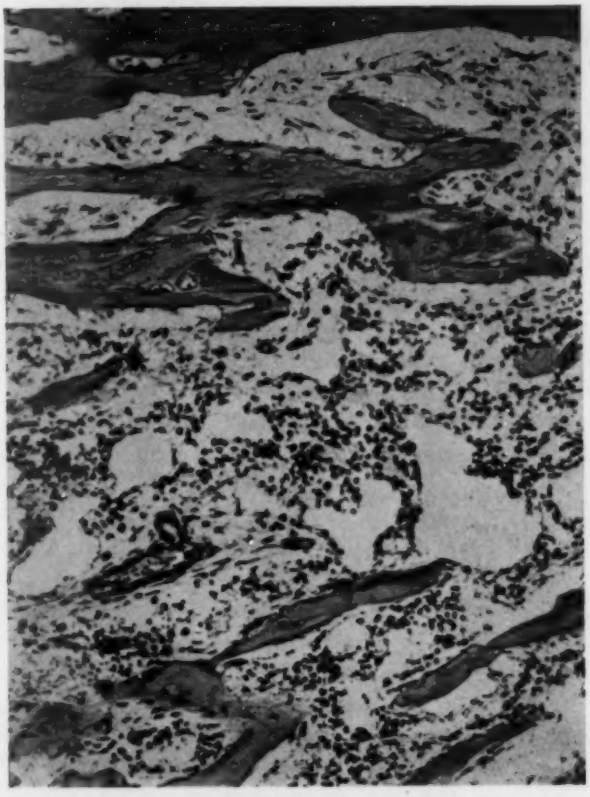


Fig. 7.—Bone marrow of a rabbit one day after birth. Note the bone trabeculae, the sheets of primitive reticular cells in the upper part of the photograph, the few fat spaces, and compare with figure 3 *B*, representing regenerating marrow as it appears in an adult animal nine days after extirpation of marrow. Note the similarity of the two marrows.

Approximation of two or more of the primitive reticular cells gave rise to fat spaces.

Presence of fat spaces was prerequisite to formation of myeloid elements in the marrow. Regeneration was not uniform. In various parts of marrow there was considerable variation in formation of fat spaces, reestablishment of normal proportions and numerical contents of cell types, and refilling of the tubular shaft of the bone.

Regeneration of marrow in adult rabbits apparently followed closely the pattern of fetal development.

Granulocytic leukocytes, erythrocytes and megakaryocytes were derived apparently from a single primitive reticular cell.

PATHOLOGIC ACTION OF DDT AND CERTAIN OF ITS ANALOGS AND DERIVATIVES

R. D. LILLIE, M.D.
Medical Director, United States Public Health Service

M. I. SMITH, M.D.
Chief Pharmacologist, United States Public Health Service
AND
E. F. STOHLMAN, B.S.
Associate Pharmacologist, United States Public Health Service
BETHESDA, MD.

SINCE the previous report [1] on the pathologic changes incident to experimental intoxication with 2,2-bis-(parachlorophenyl)-1,1,1-trichloroethane (DDT), we have studied the lesions in a small additional series of animals exposed to that compound and those in small series of animals, chiefly rabbits, exposed to various substitution products, derivatives and analogues of DDT as listed in the paper [2] dealing with the pharmacologic aspects of these compounds.

While the group of animals exposed to each compound was small, pathologic changes of interest were observed. Since some of these compounds may not be studied further at this time, it seems indicated to place on record the lesions observed with each one.

DDT

Autopsies were made on 10 rats fed DDT for a period of one year, this compound having been incorporated in their diets as follows: diet 200 (0.02 per cent DDT), 3 rats; diet 201 (0.05 per cent DDT), 3 rats; diet 202 (0.1 per cent DDT), 4 rats. Two additional rats fed DDT for two hundred and thirty days and three hundred and fifteen days died of suppurative and caseating monocytic pneumonia.

Most of the rats presented moderate to rather pronounced hypertrophy of the bronchial lymphadenoid tissue, some also focal perivascular lymphocytic infiltration of the parenchyma. Besides the 2 animals already noted, 2 more presented focal pneumonia of purulent type, and another, foci of epithelioid cell organization in alveoli. These pulmonary changes are to be regarded as infectious in nature, and the influence of the prolonged intoxication is uncertain.

From the Pathology Laboratory and the Division of Physiology, National Institute of Health, Bethesda, Md.

1. Lillie, R. D., and Smith, M. I.: Pub. Health Rep. **59**:979, 1944.

2. Smith, M. I.; Bauer, H.; Stohlman, E. F., and Lillie, R. D.: Federation Proc. **5**:203, 1946; J. Pharmacol. & Exper. Therap. **88**:359, 1946.

The heart muscle presented a variable amount of stippling of the muscle fibers with very fine to fine fat droplets, in areas or diffusely, accompanied by more or less obscuration of the cross striae. In general this change was least with diet 200, greatest with 202.

In the liver there were variable numbers of hepatic cells laden with fine fat droplets. With diet 200 this change occurred in isolated cells and in areas alternating with fat-free areas, more often in the midzones of the lobules. Similar changes were present in 1 rat dying after three hundred and fifteen days on diet 220 (also 0.02 per cent DDT). With diets 201 and 202 this fatty change was more extensive and generally involved the periportal halves of the lobules; in addition, a centrolobular cytoplasmic oxyphilia was noted. This varied from a vague diffuse change to segregation of hyaline oxyphilic masses in the central portion of the cell cytoplasm, sharply demarcated against a peripheral basophilic rim and often separated from it by a crescentic or annular vacuole. Two of the 4 rats fed diet 202 (0.1 per cent DDT) presented focal areas of coagulation necrosis of liver cells. In one of these, marginal organization was present, and organizing portal thrombi were identified, as has been noted in high level quinacrine hydrochloride intoxication (Wright and Lillie [3]; Nelson and Fitzhugh.[4]) In the other rat recrosis was recent, centrolobular, partly hemorrhagic, and variable in amount in the several lobes of the liver.

On the 0.02 and 0.05 per cent DDT diets the adrenal glands appeared normal, while on the 0.1 per cent DDT diet the medulla presented some interstitial phagocytes laden with fat droplets, as well as slight fatty changes in the parenchymal cells.

In regard to the kidney, focal chronic nephritis and pyelonephritis were observed in about half of the rats, without relation to dosage. Usually they were minor in extent, the most severe occurring in a rat whose diet contained 0.02 per cent DDT. Outside of focal areas of atrophy there were some diffuse degenerative changes affecting chiefly the deep or proximal convoluted tubules. The epithelium of these tubules was swollen and finely granular, and its radial striation more or less obscured, especially with the 0.05 and 0.1 per cent DDT levels. Fine fat droplets were present in the base of the epithelium in all rats fed the 0.1 per cent DDT diet, and in one of each of the groups whose diets contained the lower DDT percentages.

The foregoing changes are similar in nature to those previously described by us and by Nelson and co-workers [5] for rats with shorter periods of exposure.

---

3. Wright, C. I., and Lillie, R. D.: Pub. Health Rep. **58**:1242, 1943.
4. Nelson, A. A., and Fitzhugh, O. G.: Federation Proc. **3**:91, 1944.
5. Nelson, A. A.; Draize, J. H.; Woodard, G.; Fitzhugh, O. G.; Smith, R. B., Jr., and Calvery, H. O.: Pub. Health Rep. **59**:1009, 1944.

As before,[1] the hepatic alterations in the 10 additional cats studied were chiefly varying grades of fatty degeneration, perhaps more often midzonal in location, and entirely of fine droplet type. Only in 2 cats was patchy cytoplasmic oxyphilia of hepatic cells observed; distinct hyaline globules were not formed, and no cellular necrosis was noted. The periods of exposure of these cats varied from eighteen to sixty-six days at dosage levels of 5 to 50, usually 5 to 10 mg., per kilogram daily.[6] Several had presented fairly severe tremors for several days or longer before death.

The cats presented about the normal amount of fat storage in renal epithelium, and in about half there was irregular cortical tubular distention with or without oxyphilic foamy or globular exudate in the lumens. In 7 cats the spleen was essentially normal except for slight to moderate

Dosage of DDT, Survival Time and Hepatic Lesions Produced in Rabbits Given DDT by Stomach Tube

Rabbit No.	Dosage	Total Survival Time	Manner of Death	Hepatic Lesions: Necrosis	Hepatic Lesions: Hyaline Degeneration
20	9 doses of 25 mg. per Kg. = 225 mg. per Kg....	13	Died	—	—
18	1 dose of 400 mg. per Kg.....	15	Died	—	+
206	40 doses of 50 mg. per Kg. = 2 Gm. per Kg....	46	Died	+	—
52	7 doses, then 30 days' rest, then 13 doses of 100 mg. per Kg.....	60	Died	+*	—
53	19 doses of 100 mg. and single doses of 240 and 250 mg. per Kg.....	75	Died	++	+
9	2 doses of 400 and 300 mg. per Kg., 2 months' rest, then 1 dose of 400 mg. per Kg. and animal killed 4 days later.....	83	Killed	++	—

* This animal presented incipient cirrhosis.

hemosiderosis in 5 of them. Three of these 5 also showed some siderosis of Kupffer cells. The heart showed fine droplet fatty degeneration of a variable minority of muscle fibers.

The brain and the spinal cord presented pericellular vacuolation about anterior horn cells and sometimes also in motor nuclei of the medulla and higher areas of the brain stem in the 3 cats studied. In one of these, fine fat droplets appeared in many anterior horn cells, and there was partial tigrolysis. This animal had had tremors and progressive paralysis for six days before it was killed.

The liver was studied in 6 rabbits treated with DDT for thirteen to eighty-three days (see table for dosage). The first (no. 20) was without hepatic change, the second (no. 18) presented centrolobular fatty degeneration and hyaline oxyphilic cytoplasmic globules (fifteen days)

6. Smith, M. I., and Stohlman, E. F.: Pub. Health Rep. **60**:289, 1945, tables 4 and 6.

and the remaining 4 (forty-six to eighty-three days) had coagulation necrosis of variable extent, both midzonal and centrolobular. The hyaline oxyphilic cytoplasmic inclusions were present in surviving areas in one of these (no. 53). In another (no. 52) sparse fibroblastic trabeculation was developing and was segregating nodules of large basophilic liver cells. The trabeculae included also more numerous foamy (fatty?) liver cells, a little collagen, a few multinucleated giant cells and some hemosiderin-laden phagocytes. Necrosis was distinctly focal in this liver.

The kidneys and the adrenal glands presented no significant changes, the spleen moderate to rather pronounced hemosiderosis, the heart a little patchy fatty degeneration of muscle fibers.

In the hepatic hyaline degeneration and necrosis, this series is similar to those previously studied. The occurrence of an example of apparent incipient hepatic cirrhosis in conjunction with the necrosis is of interest.

DBrDT

Autopsies were made on 8 rabbits after administration of the dibromodiphenyl analogue of DDT, or DBrDT. All died in four to twenty-two days. All presented more or less extensive necrosis of hepatic cells, varying from necrosis of isolated cells only in 2 animals to large anastomosing or confluent areas of coagulative necrosis, to hemorrhagic and fibrinoid variants of the latter and to areas of congested and depleted hepatic reticulum. As with DDT in this species, there were found also, but only occasionally, focal areas of greatly swollen, highly vacuolated, fat-free liver cells. Hyaline oxyphilic cytoplasmic masses surrounded by basophilic cytoplasm, which directly adjoined them or was separated from them by annular and crescentic vacuoles, were also found in liver cells in 4 rabbits. There were also variable amounts of fine droplet fatty degeneration of liver cells. Fatty and hyaline degeneration as well as necrosis tended to occur more in the centers and midzones of the lobules. Leukocytic invasion of necrotic foci was prominent in 3 rabbits but did not involve all foci even in these.

Four of the rabbits presented more or less pronounced congestion of the lung and serous to hemorrhagic exudate in the alveoli. The heart exhibited varying degrees of fine droplet fatty degeneration of muscle fibers. The kidneys presented more or less cloudiness and swelling of the convoluted tubules, often with slight to moderate deposition of fine fat droplets in the bases of the epithelial cells. In some animals the epithelium of loop tubules was also fatty, and in some numbers of fat phagocytes or fibroblasts laden with fine fat droplets were present in the stroma of the renal pyramids.

The spleen regularly showed a moderate amount of hemosiderosis, including both the basophilic and nonbasophilic granular types and in some animals diffuse iron-staining of cytoplasm of both littoral and free

phagocytes. In the liver Kupffer cells were iron free; there was no siderosis of renal epithelium, and hemoglobin casts were not observed.

The adrenal glands were normal, with fatty cortex and intact medulla.

DDD′

Autopsies were made on 8 rabbits which had been given 4,4′-dichlorophenyl-dichloroethylene (DDD′). Six died at five to thirty-two days; 2 were killed at thirty-two days.

The liver presented an inconstant slight to moderate fine fat droplet degeneration of hepatic cells. One rabbit had hyaline oxyphilic masses and globules in the otherwise basophilic cytoplasm of liver cells in the centers of the lobules; another showed foci of hydropic degeneration in which were included some coagulated necrotic liver cells.

The spleen presented moderate to rather striking iron-positive pigmentation of macrophages and littoral phagocytes in the pulp. The pigment was of both the diffuse and the granular type.

In the kidneys there was some deposition of fine fat droplets in the epithelium of corticomedullary straight tubules, while cortical convoluted tubules showed some cloudiness and swelling of the epithelium but no fat.

The heart muscle was normal and fat free in the 2 rabbits killed at thirty-two days, while in 4 others it showed slight to moderate fatty degeneration.

Pulmonary congestion and edema were present in 2 rabbits, empyema in a third and focal perivascular lymphocytic infiltration in the rest . The pulmonary congestion and edema were probably significant; the other changes were almost surely intercurrent.

Focal hemorrhage was present in the adrenal cortex in 1 rabbit (five days), focal karyorrhectic purulent infiltration in another, while in all the animals the adrenal cortex was moderately to heavily laden with lipid substances. The medulla was normal in all.

Two rats dying three and a half days after a single dose of 1.5 Gm. per kilogram presented moderate fatty changes in liver cells, moderate to marked fatty degeneration of renal convoluted tubules with less involvement of loop tubules, moderate and rather extensive fatty degeneration of the heart muscle, much phagocytosed fat and a moderate amount of hemosiderin in the splenic pulp and in the follicular littoral cells, and normal adrenal glands.

DDD

Eight more rabbits were given 2,2-bis-(parachlorophenyl)-1,1-dichloroethane (DDD). In this series 4 rabbits died and 4 were killed at intervals of three to thirty-nine days. The liver presented inconstant and relatively slight patchy fatty degeneration. Foci of necrosis were present

in 3 rabbits, but in one the single lesion was old and encapsulated in fibrous tissue after only three days' exposure; in another a single recent focus of coagulative necrosis was present after a thirty-nine day exposure; in the third the multiple foci graded into epithelioid granulomas. On evaluation of these lesions it is believed that those in the first 2 rabbits may be excluded as not significant; in regard to the lesions in the third the question of significance must be left open.

The renal convoluted tubules were generally cloudy, perhaps swollen, but fat free. Edema of the renal pelvic fat was present in the 4 rabbits which survived over thirty days. Patchy fatty degeneration of the heart muscle was noted in 4 of the 8 rabbits, and in 1 of the 4 there was an organizing area of necrosis. The splenic pulp generally presented impressive siderosis, the pigment occurring more as diffuse iron staining of the cytoplasm of macrophages and littoral cells, but including granular hemosiderin as well, in all animals.

There were congestion and slight edema of the lung in 1 rabbit, edema, purulent bronchitis and focal purulent pneumonia in 1, and no significant pulmonary lesions in 4. The adrenal glands were regularly normal, with lipid depletion of the glomerular zone in 2 of 7. In 1 rabbit a focal area of gastric hemorrhage and edema was encountered; in 1 there was acute ulcerative ileitis. The significance of these gastrointestinal lesions is questionable.

One rat died three and a half days after a single dose of 2.5 Gm. per kilogram and another twelve days after a single dose of 3 Gm. per kilogram. They presented moderate fatty degeneration of the liver, marked fatty degeneration of renal convoluted tubules, much phagocytosis of fat and moderate hemosiderosis in the splenic pulp and follicular littoral cells, some fatty degeneration of medulla cells in the adrenal glands and, in a single rat only, patchy fatty degeneration of heart muscle.

DDM

Autopsies were made on 5 rabbits given nineteen to thirty-two doses of 50 mg. per kilogram of bis-(parachlorophenyl)-methane (DDM) over periods of twenty-two to thirty-seven days. One rabbit died with purulent bronchitis, pulmonary edema, purulent pleurisy and pericarditis and centrolobular fatty degeneration and necrosis of the liver. In the 4 rabbits remaining, the liver presented only slight fatty changes in parenchymal cells and some phagocytosis of fat in littoral cells. The kidneys and the adrenal glands were normal. The heart muscle was usually fat free; 1 rabbit showed involvement of a few fibers. Congestion and edema of the lung occurred in 2 rabbits. The splenic pulp presented definite hemosiderosis both as granules and as diffuse iron staining of the cytoplasm of littoral cells and free macrophages. No gastric lesions were observed.

DT

Autopsies were made on 11 rabbits which had received over periods of two to thirty-eight days aggregate doses of 200 mg. to 1.6 Gm. per kilogram of 2,2-diphenyl-1,1,l-trichloroethane (DT).

In the liver fatty changes were generally slight or lacking. Focal necrosis, coagulative, fibrinous or purulent, was seen in 4 rabbits, 3 dying in two to four days, and 1 in twenty-six days, after daily doses of 100 mg. per kilogram. Oxyphilia of the central part of the cytoplasm of hepatic cells, without segregation of distinct hyaline globules, appeared in 3 rabbits killed or dying on the tenth, tenth and sixteenth days. The 4 animals killed after thirty-one to thirty-eight days showed slight fatty changes as the only hepatic alteration.

The epithelium of the renal convoluted tubules was often cloudy, less often swollen, and in 5 animals only of the 10 studied was there slight patchy fatty degeneration. Edema of the pelvic fat was noted in 2 rabbits.

Focal hemorrhage appeared in the fatty adrenal cortex in 1 of 8 rabbits. In the remaining 7 the adrenal glands were normal.

One rabbit had focal purulent and necrosing fibrinous pneumonia and mediastinitis, fibrinous pericarditis and focal myocardial hemorrhage and edema. In 3 other rabbits pulmonary congestion and edema were noted, while no significant lesions appeared in 3. Slight to fairly prominent focal to diffuse fine droplet fatty degeneration of heart muscle appeared in 6 of 8 rabbits.

The splenic pulp presented moderate hemosiderosis of littoral cells and of free macrophages. The pigment was of both granular and diffuse types in 5 rabbits, granular only in 2 and slight and diffuse only in 1.

One rabbit had subacute ulcerative typhlitis with glandular hyperplasia, hemorrhage, edema, exudation of fibrin and local peritonitis and in one area of the stomach necrosis, edema, hemorrhage and calcification of muscle. In this animal there was also subacute interstitial nephritis. The significance of these lesions in relation to DT is not clear.

DDA

P,p′-dichlorodiphenylacetic acid (DDA) was given intravenously to 6 rabbits, the sodium salt being used in aqueous solution. These rabbits received single doses respectively of 200, 150, 100, 150, 150 and 100 mg. per kilogram and died one and a half, three, four, five and a half, six and seven and a half days later. (The lethal dose [50 per cent] of DDA when it is injected intravenously into rabbits is between 100 and 150 mg. per kilogram.)

In 1 rabbit the liver was normal except for slight hemosiderosis of littoral cells; in 2 there were fairly definite congestion and slight to moderate fatty degeneration of hepatic cells; in 2, isolated hepatic cells,

respectively few and numerous, exhibited cytoplasmic oxyphilia grading over to coagulative necrosis and to the formation of hyaline oxyphilic cytoplasmic inclusion bodies. Of three blocks from the liver of the sixth rabbit, there was confluent or an astomosing midzonal coagulative necrosis of hepatic parenchyma generally in one and focally in another; the third block showed none. In the necrotic areas centers of lobules were sometimes involved as well, the included capillaries were engorged with red corpuscles, and marginal surviving liver cells were laden with fine fat droplets.

The kidney regularly presented irregular dilatation of convoluted tubules and more or less numerous casts within them. The casts were sometimes hyaline and basophilic, more often oxyphilic and hyaline or finely granular, and frequently stained orange pink with eosin in the same tint as red corpuscles within vessels. The last type were hyaline or finely to coarsely granular or even composed apparently of erythrocytes. Pyramidal tubules often contained similar hemoglobin casts. Otherwise, convoluted tubules often presented normal lining epithelium or only focal deposition of fine fat droplets in the epithelium. In the 2 rabbits surviving to six and seven and a half days there were respectively numerous calcified necrotic epithelial cells in the convoluted tubules and a few small concentrically laminated calcareous bodies in the lumens. Albuminuria is a constant finding in rabbits after intravenous administration of DDA.

Three rabbits presented pulmonary congestion and a moderate amount of serous exudate in the alveoli. In the others no significant pulmonary lesions were observed.

The heart presented patchy to diffuse deposition of fine fat droplets in the muscle fibers, sometimes of high grade, in the 5 rabbits surviving three to seven and a half days.

The adrenal cortex was generally fatty in the inner half, and sometimes throughout. The medulla appeared normal.

The splenic pulp generally contained a considerable amount of blood and moderate numbers of lymphocytes. In the rabbit which died in thirty-six hours the iron reaction was negative, while in the rest of the animals considerable amounts of hemosiderin were present in swollen littoral cells and free phagocytes. The follicles were small and relatively inactive.

Six other rabbits received DDA by stomach tube, 50 mg. per kilogram daily. Two of them died at nine and twenty days; 3 were killed on the thirty-eighth day and 1 on the fifty-second.

The rabbit dying at twenty days presented purulent bronchitis and pneumonia, focal purulent and coagulative necrosis in the heart muscle, focal necrosis with marginal organization in the liver, necrosis, regeneration, hyaline casts and slight fatty degeneration in the kidney and marked splenic hemosiderosis.

Otherwise, in this series only slight fine fat droplet deposition was noted in hepatic and sometimes in Kupffer cells. The kidneys showed areas of tubular dilatation and some epithelial or hyaline casts in 4 of the 5 remaining rabbits, usually without fatty changes. Slight to moderate patchy fine droplet fatty degeneration of the heart muscle appeared in 3 of the 5 rabbits. A pulmonary infarct was present in 1 rabbit, bronchitis and edema in 1 and no pulmonary lesions in 3. The adrenal cortex was fatty in all 5; the medulla, normal. The splenic pulp in 3 of 4 rabbits presented moderate pigmentation by iron-positive and often also iron-negative pigment.

Altogether, the rabbits given DDA by stomach tube presented much less striking lesions than those dying after intravenous administration, but blood destruction again seems to have been the principal injury.

Three rats were killed six days after intravenous injection of 100 mg. per kilogram of DDA. They showed no significant lesions of the liver, the adrenal glands or the lungs. Focal renal necrosis was present in 1 rat, and slight cloudy swelling in 1. In all the spleen presented moderate erythropoietic and myelopoietic activity, moderate numbers of megakaryocytes and little or no hemosiderin. All were killed in six days.

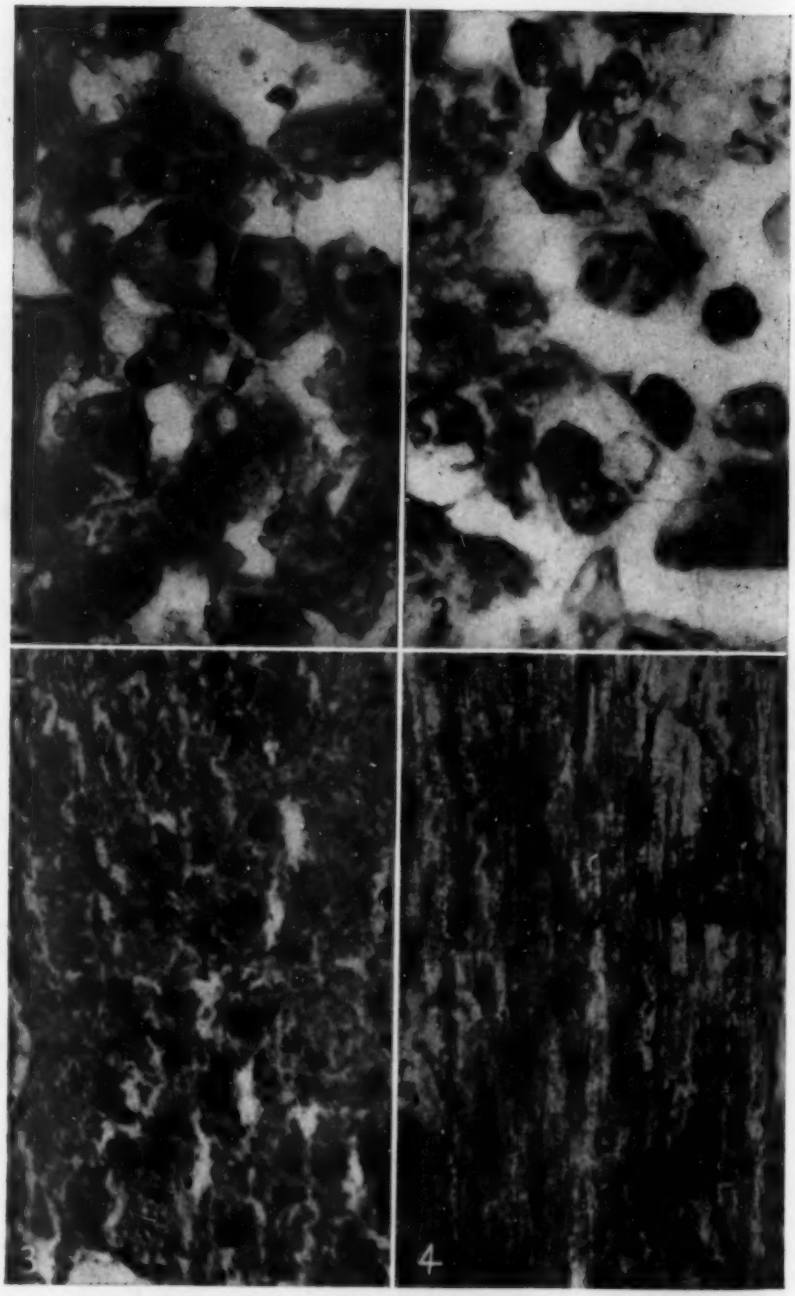
DE

Five rabbits were given twenty-one to thirty-four doses of 50 mg. per kilogram of diphenylethane (DE) over periods of twenty-three to thirty-nine days. Renal convoluted tubules were radially striated and fat free. The adrenal medulla was normal; the cortex heavily laden with lipid. One rabbit had an abscess of the adrenal cortex, probably metastatic from the chronic necrosing pneumonia noted in one lung. Another rabbit presented pulmonary edema, while in 3 there were no pulmonary lesions. Patchy fatty degeneration of the heart muscle was noted in 1 rabbit but not in 3 others. Scattered liver cells and Kupffer cells contained small amounts of fat in fine droplets, and the epithelium of small bile ducts was often moderately laden with fine fat droplets. In 1 rabbit a pyogenic ulcer of the stomach and focal cholecystitis were present. In 4 rabbits the splenic pulp presented moderately severe hemosiderosis, the pigment appearing both in granular and diffuse form in littoral cells and in free macrophages.

DA

Four rabbits were given thirty to thirty-three doses of 50 mg. per kilogram of diphenylacetic acid (DA) over periods of thirty-five to forty-one days.

Three animals presented centrolobular accumulation of coarse and medium fat globules in liver cells, moderate in 2 rabbits and slight in 1. The remaining rabbit showed none. In 1 rabbit examined three hours post mortem, some epithelial desquamation and hyaline casts were found



(See legends on opposite page)

in renal convoluted tubules; otherwise the renal parenchyma was normal and fat free. One rabbit had ulcerative pyelitis with a stone. Fatty degeneration of the heart muscle was present in 3 rabbits, slight in 2, and rather marked in 1; the remaining rabbit showed none. Moderate hemosiderosis of pulp phagocytes and littoral cells was noted in the spleen in all 4. The adrenal cortex was fatty; the medulla, normal. Congestion and serous alveolar exudation were present in the lungs in 2 rabbits but not in the other 2. The stomach and the gallbladder were normal.

DDK′ AND DDK

Dichloro-benzophenone was given to 9 rabbits, 6 receiving the asymmetric 2,4′-compound (DDK′), 3 the symmetric 4,4′- or para-, para′-isomer (DDK). Three rabbits received four doses of 400 mg. per kilogram of DDK′ and died on the fourth day. Two had an additional 800 mg. and died on the ninth day. One received seven doses of 400 mg. per kilogram and survived ten and a half days.

With the 2,4′-isomer the lungs presented more or less marked congestion and edema. Alveolar exudate was mainly serous and faintly to moderately eosinophilic, and in the animals dying in four days it contained moderate numbers of desquamated rounded epithelial cells. Attached swollen alveolar epithelium was conspicuous in 1 rabbit.

With 4,4′-dichloro-benzophenone there were no pulmonary lesions in 2 rabbits, while lobar pneumonia was found in the third.

With both isomeres the heart exhibited slight to moderate, patchy to diffuse deposition of fine fat droplets in muscle fibers. The adrenal cortex was more or less laden with fat, especially in the inner half, and the medulla was essentially normal. In the spleen slight to moderate hemosiderosis of pulp littoral cells was the principal finding. Iron-positive pigmentation occurred both as diffuse cytoplasmic staining and as typical granular hemosiderin.

The renal convoluted tubules were generally more or less cloudy and swollen. Fatty changes were inconstant and of slight to moderate grade when present. Necrosis and desquamation of epithelial cells were noted in 1 rabbit with each isomere, hyaline casts in dilated tubules in 2 others of each group. In 3 rabbits receiving the 2,4′-dichloro-benzophenone, casts and epithelial necrosis were absent, but orange-staining

---

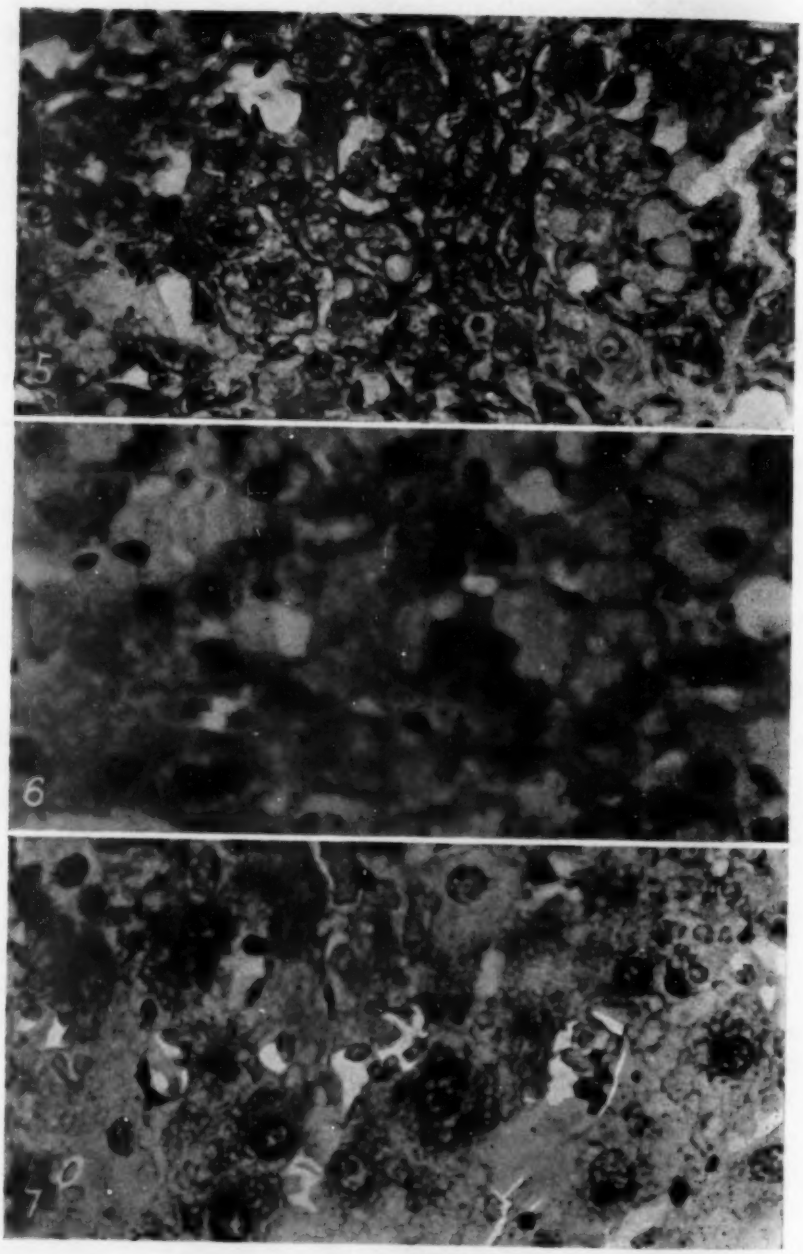
EXPLANATION OF FIGURES 1 TO 4

Fig. 1.—Hyaline liver of a rabbit fed DT for ten days; azure eosin; × 700.

Fig. 2.—Cytopyknosis and necrosis of isolated hepatic cells of a rat fed a single dose of DMDT and killed seven days later; azure eosin; × 1,000.

Fig. 3.—Hemosiderosis of the spleen of a rabbit fed DDD and examined thirty-eight days later; acetic ferrocyanide safranin; × 250.

Fig. 4.—Fatty degeneration of the heart muscle of a rabbit given DDD′ and examined thirty-two days later; hematoxylin oil red O; × 250.



(See legends on opposite page)

eosinophilic casts were noted in pyramidal tubules in 1 rabbit receiving this compound. Fine fat droplets had been deposited in the epithelium of the loop tubules in the corticomedullary zone in all 6 rabbits with the 2,4′-isomere and in 1 of the 3 with the 4,4′-compound.

The pathologic alterations of the liver were unfortunately complicated by the presence of definite or probable recent or old coccidiosis in nearly all of these animals. Otherwise minor fatty changes were seen in all the rabbits. The 1 rabbit in which the deposition of fine droplets of fat in liver cells was of severe grade presented also patchy areas of swollen hydropic liver cells enclosing a few isolated coagulated necrotic cells, as in DDT poisoning. This was the latest survivor in the 2,4′-dichlorobenzophenone group.

DMDT

When p,p′-dimethoxydiphenyl-trichloroethane (DMDT) was administered to 9 rats by stomach tube in single doses of 2 to 8 Gm. per kilogram, only 1 rat died, death occurring after five days. This rat received the lowest dose of the group. In this animal the liver presented many isolated hepatic cells in varying stages of coagulative necrosis, from simple cytoplasmic oxyphilia to rounded, shrunken oxyphilic globules with or without contained nuclear fragments. There were also focal areas of engorged and depleted parenchyma, in which there were often isolated surviving and necrosing liver cells, rarely coherent coagulated necrotic liver cells. Fatty degeneration of liver cells occurred irregularly, especially adjoining areas of necrosis. Two other rats, killed on the seventh day, showed necrosis of isolated liver cells. One of these had a considerable amount of epithelioid cell proliferation about necrotic cells and independently, to form small granulomas. Small granulomas were seen also in another rat, centering about clumps of fragmenting leukocytes. These are of dubious significance. Otherwise, the remaining rats showed fatty degeneration of scattered isolated liver cells.

In this same series, a material amount of renal fatty degeneration and of fatty degeneration of heart muscle was evident in the same rat which showed the greatest hepatic damage. In the other rats there was often focal interstitial nephritis, such as one often finds in rats, and small foci of interstitial myocarditis. In 1 rat there was a bulky area of fatty degeneration and atrophy of the heart muscle, with extensive

---

EXPLANATION OF FIGURES 5 TO 7

Fig. 5.—Destruction of hepatic cells and beginning interstitial fibrosis and trabeculation in a rabbit fed DDT for sixty days; Van Gieson stain; × 250.

Fig. 6.—Hyaline degeneration of the liver of a rabbit given DDD′ and examined thirty-one days later; azure eosin; × 700.

Fig. 7.—Necrosis of isolated cells of the liver of a rabbit which died thirty-six hours after receiving NaDDA intravenously; azure eosin; × 700.

interstitial proliferation and lymphoid cell infiltration. This involved the base of the left ventricle. This lesion was regarded as intercurrent.

The lungs presented more or less frequent interstitial and perivascular lymphocytic infiltration, hyperplasia of bronchial lymphoid follicles and

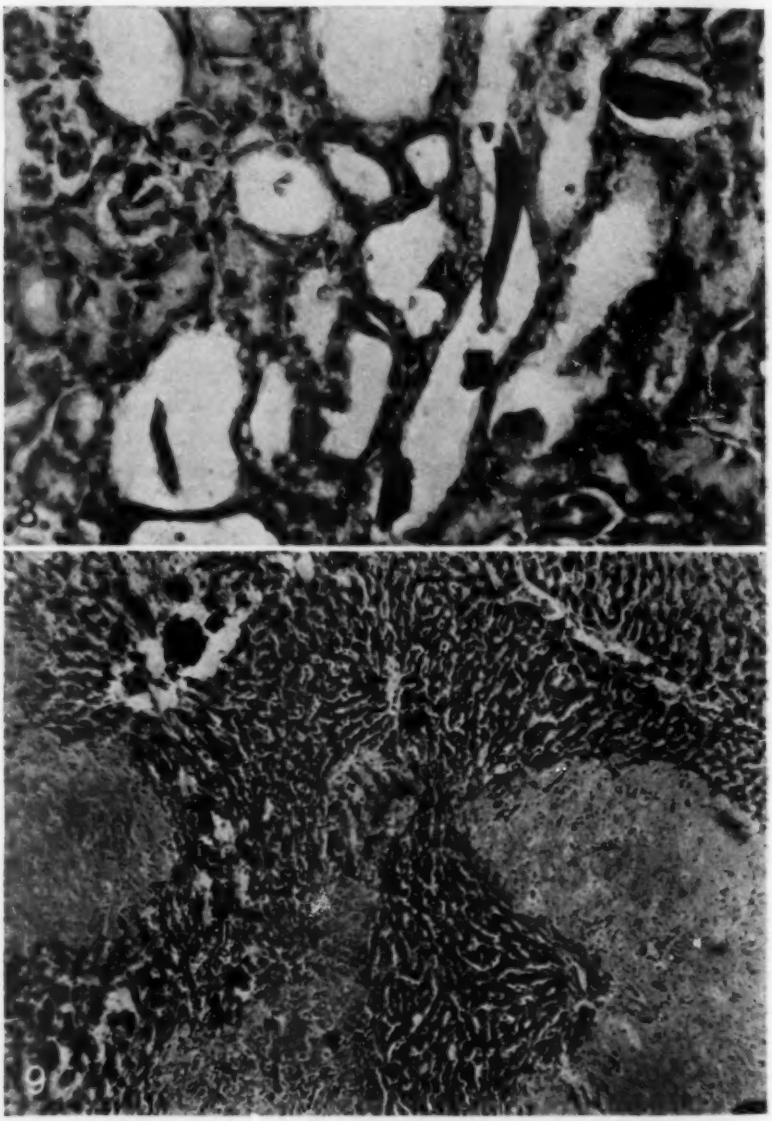


Fig. 8.—Hemoglobin casts in the kidney of a rabbit that died five and a half days after receiving NaDDA intravenously; azure eosin; × 250.

Fig. 9.—Necrosis of the liver of a rabbit which died six days after receiving NaDDA intravenously; azure eosin; × 70.

patchy atelectasis. In 1 rat there was focal organizing bronchopneumonia. Such lesions are not uncommon in rats, especially after feedings by stomach tube.

Moderate splenic myelosis was present in 3 rats, pulpal hemosiderosis in 3 others and some follicular hyperplasia in 2 others. Altogether, there were no consistent alterations.

Four rabbits were given daily doses of DMDT at 200 mg. per kilograms by stomach tube. They received respectively five, six, nine and fifteen doses and died in five, six, ten and twenty days.

The first of these presented ulcerative bronchitis, seropurulent pneumonia and pulmonary edema, also some parenchymatous degeneration of the kidney and fatty degeneration of the heart muscle and of the hepatic parenchyma, with vague areas of hepatic cell oxyphilia and karyopyknosis suggesting incipient necrosis. Pulmonary edema and interstitial pneumonitis, fatty degeneration of the liver and parenchymatous degeneration of the kidney were the principal findings in the second. In the third there were pulmonary congestion, purulent bronchitis and bronchopneumonia, necrosis of the gallbladder and of adjacent liver tissue (but not of other parts of the liver), parenchymatous and fatty degeneration of the kidney with some epithelial necrosis, desquamation and perhaps early calcification, and catarrhal enterocolitis. In the fourth rabbit again there were edema of the lungs, parenchymatous degeneration, epithelial necrosis and desquamation of epithelium of the kidney, slight centrolobular fatty degeneration of the liver and focal myocardial necrosis with calcification. The second and fourth rabbits presented moderate hemosiderosis of the splenic pulp.

The pulmonary changes were probably due to the repeated feedings by stomach tube and consequent accidental aspiration. Otherwise, the renal degenerative changes appear to be the most consistent alterations. The number of animals studied is small, however.

SUMMARY

Further studies of the cat brain and cord in DDT poisoning revealed moderate fatty degeneration of nerve cells as a probably significant, though infrequent finding. In 1 rabbit incipient trabeculation and nodular hyperplasia of the liver developed on the basis of distinctly focal necrosis, indicating the possibility of hepatic cirrhosis from more prolonged exposures. Nelson and co-workers [5] have also noted regenerative hyperplasia of the liver in combination with necrosis.

The hepatic lesions induced by dibromodiphenyl-trichloroethane closely resemble those of DDT poisoning: coagulative necrosis and hyaline, hydropic and fatty degeneration. Of these changes, only the fatty degeneration recurred with any consistency with other derivatives, and it was often slight in grade. With DDD and DDD′ it was slight and

inconstant, with DDM slight, with DT slight or lacking, with DDA slight after feeding by stomach tube and moderate after intravenous injection, with DE slight, with DA variable, with DDK and DDK′ slight to moderate. Focal and patchy necrosis, other than clearly intercurrent lesions, were present in 1 rabbit each in the DDD, DDD′ and DDK′ series, in 4 of the DT series of 11, in all 8 given DBrDT and in 3 of 6 given DDA intravenously. Often these lesions were few, and sometimes they were apparently purulent in character. Their relation to the experimental condition cannot be excluded on the latter account, since hepatic necroses due to DDT are also often heavily infiltrated by leukocytes.

Hyaline degeneration of the cytoplasm of liver cells is probably more definitely a toxic effect. It was found in 3 of 6 DDT rabbits, in 4 of 8 DBrDT, 1 of 8 DDD′ and 3 of 11 DDM rabbits. The focal hydropic degeneration previously described in DDT poisoning occurred in 5 rabbits—3 on DBrDT, 1 each on DDD′ and DDK′—and the foci, as before, often contained a few coagulated necrotic cells as well.

Other lesions of interest in the present series are the renal and the splenic changes. More or less consistently throughout the series of compounds used, moderate hemosiderosis of the splenic pulp was produced. As a part of the same picture, definite hemoglobinuric nephrosis was produced by DDA when this was administered by the intravenous route. Less pronounced nephrosis, without obvious hemoglobinuria, appeared also in most of the animals given this compound by stomach tube. Otherwise moderate cloudy swelling and fatty degeneration of convoluted and loop tubules appeared with DBrDT—less with DMDT and with DT. There was an instance of hemoglobinuric nephrosis with DDK′. Fatty degeneration of loop tubules was the principal change with DDD, DDD′, DDK and DDK′, while no renal damage was evident with DE, DA, DDM or DDT in rabbits.

Congestion and edema of the lungs were observed in 4 or 5 lungs studied histologically with DBrDT, 1 of 2 with DDT, 1 of 6 with DDD, 2 of 8 with DDD′, 2 of 5 with DDM, 1 of 5 with DDA administered by stomach tube, 1 of 4 with DDA injected intravenously, 1 of 5 with DE, 2 of 4 with DA, 4 of 7 with DT, all of 6 with DDK′ and none of 3 with DDK. Purulent pneumonia was seen in 4 rabbits which had received respectively DDD, DDD′, DE and DDK. All rabbits given DMDT had either pneumonia or pulmonary edema.

It is necessary to consider aspiration secondary to feeding by stomach tube as a possible factor in the causation of these pulmonary changes.

REPAIR IN THE SKIN OF GUINEA PIGS SUBSEQUENT TO APPLICATIONS OF 20-METHYLCHOLANTHRENE

MARTIN SILBERBERG, M.D.
AND
RUTH SILBERBERG, M.D.
ST. LOUIS

THAT the skin of guinea pigs is resistant to the action of polycyclic hydrocarbons is manifest in the low incidence of cancers following administration of these substances. Fibrosarcoma and liposarcoma have been obtained in the subcutaneous tissues.[1] Carcinoma has not as yet been produced. This does not preclude the possibility that the epidermis of the guinea pig may be able to react in some way to the polycyclic hydrocarbons applied. In mice, prolonged cutaneous application of benzpyrene and methylcholanthrene previous to the making of a wound caused increased proliferation of the epidermis during repair. However, this intensified growth was not accompanied by more rapid epithelization of the defects; on the contrary, the regenerating epithelium was delayed in its migration over the surface of the wound.[2] The present investigation was undertaken in order to determine whether similar effects might be observed in the repair of the skin of the guinea pigs. Moreover, we intended to study the response of the carcinogen-treated epidermis in different sites, varying in thickness, such as those found in the flank and the ear, in the same guinea pig.[3]

MATERIAL AND METHODS

Thirty-two male guinea pigs weighing 180 to 220 Gm. at the beginning of the observation were used. The right ears and the right flanks of all animals served as test areas. The hair was carefully clipped before the painting was started, and clipping was repeated at frequent intervals, whenever the hair had

This investigation was aided by the David May-Florence G. May Fund.

From the Snodgras Laboratory of Pathology, City Hospital, and the Pathological Laboratory, Jewish Hospital.

1. Haagensen, C. D., and Krehbiel, O. F.: Am. J. Cancer **27**:474, 1936. Shear, M. J.: ibid. **33**:499, 1938. Schabad, L. M.: Arch. biol. sc. **51**:112, 1938. Warren, S., and Gates, O.: Cancer Research **1**:65, 1941. Shimkin, M. B.: J. Nat. Inst. Cancer **1**:707, 1941.

2. Silberberg, M., and Silberberg, R.: Am. J. Path. **20**:809, 1944; Arch. Path. **42**:193, 1946.

3. Loeb, L., and Addison, W. H. F.: Arch. f. Entwcklngsmechn. d. Org. **37**:635, 1913. Loeb, L., and Spain, K. C.: J. Exper. Med. **25**:107, 1916. Loeb, L.: J. M. Research **41**:247, 1919.

regrown. Three times weekly the skin was painted with a 0.3 per cent solution of 20-methylcholanthrene dissolved in benzene, the solution being applied in one stroke with a no. 6 camel's hair brush. The left ears and the left flanks served as controls. In 16 of the animals the left ear and the left flank remained untreated, to be used later for the making of control wounds. In the 16 remaining guinea pigs the left flank and the left ear were painted with benzene alone for the same periods as the opposite side with methylcholanthrene. Painting was continued for one-half month, one month, two months or three months. After each of these periods wounds were made in subgroups of 8 animals. An area 4 mm. in diameter was marked with a round stencil, and the skin, with some underlying tissue, was excised. Thus in each animal four wounds were made, one in each ear and one in each flank. On the right side each guinea pig had two wounds which were under the influence of the previously applied carcinogen. On the left side each animal carried control wounds, made either in normal skin or in epidermis previously treated with benzene. Repair was allowed to take place for three, five, eight or ten days. At the end of each experimental period the wounds, with the surrounding skin and subcutis, were removed, fixed in solution of formaldehyde, U.S.P., diluted 1:10, embedded in paraffin, cut serially and stained with hematoxylin and eosin.

The epithelium at some distance from the line of excision (distant epithelium), that at the margin of the wound (marginal epithelium) and the newly formed epithelium growing in a tonguelike fashion into the defect (new epithelium) were studied separately. In each case the lengths of the epithelial tongues were measured. The size of the cells in each of the various areas was determined, and the epithelial cell layers and the mitoses in the epidermal cells were counted.

GROSS OBSERVATIONS

Only in a few animals after application of methylcholanthrene for three months was some epilation noted. During the operation the wounds made in the areas previously treated with benzene or methylcholanthrene bled more profusely than those made in untreated skin.

HISTOLOGIC OBSERVATIONS

Closure of the Wounds.

All wounds, whether made in untreated or in painted epidermis in the ear or in the flank, were closed eight days after excision; still, the different areas of the epithelium, designated as distant, marginal and new, could be recognized as such, although the tongues had met in the center of each of the defects. At three or five days after operation the tongues varied in length considerably. No definite correlation could be established between the various experiments and the progress of epithelization. Shorter epithelial tongues usually were present if there was marked edema or cellular infiltration in the dermis or the subcutis.

Changes in the Wound Base.

Untreated Animals.—After three days of healing, the connective tissue at the base of the wound was loose, and the uppermost layer was covered with fibrin and slightly infiltrated by polymorphonuclear and

mononuclear leukocytes. Five or eight days after the making of the wound, capillaries advanced toward the surface and many fibroblasts appeared, whereas round cells and polymorphonuclear leukocytes became less numerous. Here and there a multinucleated giant cell was found. After ten days, when the wounds had closed, the tissue became fibrillar, with no evidence of an inflammatory reaction. In the ear and the flank the conditions were similar except that collagenous fibers were much more abundant in the subcutis of the flank than in that of the ear.

Benzene-Treated Animals.—After painting with benzene for one-half month or one month, the connective tissue was looser and more engorged than that in the untreated animals. This change was noted as early as three days after excision, not only in the wound base proper but also in the adjoining dermis and subcutis. The fibrils were thickened. At the floor of the defect were minute hemorrhages and much fibrin; polymorphonuclear and mononuclear leukocytes were more numerous than in the nontreated guinea pigs, and they infiltrated the deeper layers of the subcutis. After five or eight days, numerous engorged capillaries, accompanied by fibroblasts and a few multinucleated giant cells, advanced toward the surface. Polymorphonuclear leukocytes and lymphocytes were scanty. After ten days a densely fibrillar connective tissue was found in the former wound base. As in nontreated skin, the collagenous fibrils were by far more abundant in the flank than in the ear. The changes described were intensified after two or three months of treatment. The swelling of the fibrils was more marked and the exudate was more abundant than after shorter periods of painting. Ten days after operation the collagenous fibers were frequently fragmented. Again, as in the untreated animals, the scar in the flank contained much more collagenous tissue than that in the ear.

Methylcholanthrene-Treated Animals.—After one-half or one month's treatment conditions in both the ear and the flank were comparable to, but more pronounced than, those seen after application of benzene alone; there were also some eosinophils and mast cells. After two or three months of painting with methylcholanthrene the changes in the connective tissue were still similar in kind but more severe than those in the corresponding benzene-treated animals. After ten days the scar was deeper, more collagenous and more vascular and still contained some inflammatory cells.

Thickness of the Epidermis.

In the untreated skin of both the ear and the flank the proportion of basal to spinous cells was 2 or 3 to 1. Under the influence of benzene, as well as under that of methylcholanthrene, this ratio shifted gradually in favor of the spinous cells. Thus, after three months of

painting there was one spinous cell found for each basal cell. As soon as the painting was discontinued, the number of spinous cells decreased again, and ten days after the making of the wound, that is, eleven days after the last painting, the usual ratio of the two cell types was restored in most instances. In table 1 the numbers of cell rows of the epithelium of the ear are presented separately from those of the flank. The data for the untreated skin are shown in columns 2 to 4. The findings after painting with benzene for one-half or one month were similar, as were those after painting for two or three months. The same was true for the figures obtained with methylcholanthrene; therefore, the one-half and one month stages (columns 5 to 10), and the two and three month stages (columns 11 to 16), are grouped together. The numbers of cell rows for the distant, the marginal and the new epithelium are given in individual columns.

Ear.—The untreated epidermis consisted of four rows of cells. During wound-healing the thickness of the marginal epithelium increased to a maximum of nine rows of cells five to eight days after excision; after the wounds were closed, it declined slowly, so that ten days after operation there were seven layers of epithelial cells at the wound margins. During the first five days the advancing epithelial tongues at their insertion were about as thick as the distant original epithelium consisting of four rows of cells. After the defects closed, the tongues were composed of six layers of cells, and they merged invisibly with the old epithelium at the line of excision.

After benzene had been applied for one-half or one month, the epidermis became slightly thickened and contained about five rows of epithelial cells. After excision of a piece of skin the thickness of the epidermis at the wound margin was not increased over that seen in untreated skin. The maximum number of epithelial cell layers was nine. However, in the benzene-treated animals the greatest thickness was present as early as three days after the making of the wound, whereas in untreated epidermis the maximum number of cell rows was seen after five days. As in the untreated skin, the height of the regenerating epithelial tongues was about the same as that of the distant epithelium, and it decreased somewhat as the tongues from both sides joined to close the defect. Treating the epidermis with benzene for two or three months did not produce any further thickening as compared with that seen after one-half or one month's application. But after excision the height of the marginal epithelium increased to ten rows and, in contrast to that of the animals treated for shorter periods, it had a tendency to increase even after the wounds had closed. The same tendency was noticeable in the epithelial tongues.

After one-half or one month's treatment with methylcholanthrene five epithelial cell layers were present in the original (distant) epithelium. At the wound margin the maximum number of cell rows was nine, as

TABLE 1.—*Number of Cell Rows in Various Areas of the Epithelium After an Excision Made in Untreated Skin and in Skin Painted with Benzene or with Methylcholanthrene Dissolved in Benzene.*

Days of Healing	Untreated Skin			Benzene ½ or 1 Month			Methylcholanthrene ½ or 1 Month			Benzene 2 or 3 Months			Methylcholanthrene 2 or 3 Months		
	Distant Epithelium	Marginal Epithelium	New Epithelium	Distant Epithelium	Marginal Epithelium	New Epithelium	Distant Epithelium	Marginal Elpthelium	New Epithelium	Distant Epithelium	Marginal Epithelium	New Epithelium	Distant Epithelium	Marginal Epithelium	New Epithelium
							Ear								
3	4	6	4	5	9	5	5	9	5	5	8	5	6	9	5
5	4	9	4	5	8	4	5	9	5	4	9	5	5	10	5
8	4	9	5	5	7	5	5	9	5	5	9	6	5	10	8
10	4	7	6	5	6	4	5	8	6	5	10	7	5	8	6
							Flank								
3	2	6	4	4	7	5	4	7	5	4	7	4	5	7	5
5	3	7	4	3	8	4	4	8	4	4	7	5	5	9	5
8	2	7	6	4	7	4	4	8	5	4	7	6	5	8	6
10	2	6	5	5	5	5	3	5	5	3	6	3	4	6	5

TABLE 2.—*Number of Mitoses in Multiples of the Normal in Distant, Marginal and New Epithelium After an Excision Made in Untreated Epidermis and in Skin Painted with Benzene or Methylcholanthrene*

Days of Healing	Untreated Skin			Benzene ½ or 1 Month			Methylcholanthrene ½ or 1 Month			Benzene 2 or 3 Months			Methylcholanthrene 2 or 3 Months		
	Distant Epithelium	Marginal Epithelium	New Epithelium	Distant Epithelium	Marginal Epithelium	New Epithelium	Distant Epithelium	Marginal Elpthelium	New Epithelium	Distant Epithelium	Marginal Epithelium	New Epithelium	Distant Epithelium	Marginal Epithelium	New Epithelium
							Ear								
3	1	6	1.5	1.5	6	2	1.5	6	3	2	7	3	1.5	7.5	3
5	1	5	5	1.5	6	4	1	5.5	4.5	1.5	4	4	2	7	6.5
8	1	4.5	3	1	5	2	1	4.5	3	2	3.5	3.5	1.5	6	5.5
10	1	2	1.5	1	2	1	1	2	1.5	1.5	2	1.5	1.5	3	3
							Flank								
3	1	3.5	1	1.5	5	2	2	5.5	2	1.5	8	2	1.5	8	3
5	1	6	5	1.5	6	6	1	5	7	2	6	6	2	8	6
8	1	4	3.5	1	3	1.5	1	3	3	1.5	4	3	1.5	4	4
10	1	2	1	0.5	0.5	0	1	1	1	1.5	1.5	1	1	1.5	1

in the benzene series; but this "high" remained present for eight days, and there was a slight decline to eight rows of cells after ten days of healing. The epithelial tongues again were of about the same thickness as the distant epithelium. In some of the animals treated for two or three months the number of cell rows in the distant epithelium increased to six. Under the combined stimulation of the carcinogen and the wound the marginal epithelium was not higher than under the influence of benzene and the wound; but the maximum of ten layers of epithelial cells was reached after five days, and it persisted through the eighth day. The epithelial tongues showed, as in the benzene-treated and in the untreated skin, about the same height as the epithelium distant from the wound (five or six rows).

Flank.—The untreated epidermis distant from the incision had two to three rows of cells, compared with four in the ear. During the course of repair the largest number of cell rows seen at the wound margin was seven (five or eight days after operation); the new epithelium was composed of four to six rows of cells. One-half or one month's, as well as two or three months' treatment with benzene increased the number of cell layers in the distant epithelium to four or five. The marginal epithelium around the wounds made in benzene-treated skins reached a maximum thickness of eight rows of cells, and there was little difference between the groups treated with benzene for short or longer periods. The height of the new epithelium slightly exceeded that of the distant epithelium, reaching a maximum of five rows after one-half or one month and a maximum of six rows after two or three months of application. The thickness of the epidermis after one-half or one month's treatment with methylcholanthrene did not differ from that seen after application of benzene alone. Prolonged painting with methylcholanthrene evoked a reaction slightly more marked than that following the same length of application of benzene alone. The difference amounted to one row of cells in the distant, and to two rows of cells in the marginal, epithelium. The maximum of nine cell rows was present after five days, after which time the epithelium again became thinner.

Size of the Epithelial Cells.

Ear.—In the normal skin the basal cells measured 6 to 7 microns in width. Three months' application of the carcinogen increased their diameter to 7 or 8 microns. At the margin of a wound made in untreated skin the diameter of the cells was about 8 microns, and in the new epithelium cells measuring as much as 10 microns were observed, particularly during the first five days of healing. The same increase in size, with some variation, was observed in the cells under the influence of benzene and methylcholanthrene. Here and there the regenerating cells reached a diameter of 12 microns. Thus the effect of the carcinogen on cell size was far less accentuated than that of the excision.

Flank.—The basal cells were normally somewhat larger than those of the epidermis of the ear, having a width of 7 to 8 microns, compared with 6 or 7 in the latter. But under the stimulation of either the wound alone or the wound in combination with methylcholanthrene the maximum diameter did not exceed that reached under the same conditions by the naturally smaller cells of the ear.

MITOTIC COUNTS ON THE EPITHELIUM

In table 2 the numbers of mitoses in 2,000 epithelial cells are presented for the ear and for the flank. The values are means established on the basis of counts of 20,000 cells each in the distant, the marginal or the new epithelium. The number of mitoses in untreated skin was 3 or 4 in 2,000 cells for the ear and 2 or 3 in 2,000 cells for the flank.

The increases of mitoses are presented in multiples of the normal number, so as to allow a better comparison of the counts arrived at under the varying experimental conditions and in the two locations studied. In table 2 the same arrangement is followed as in table 1, and the results obtained after one-half or one month and those established after two or three months are grouped together. The effects of the one-half or one month's treatment were slight at best and do not warrant separate discussion. The changes in the mitotic counts after two or three months' painting were, on the whole, more marked than after short treatment and are illustrated in charts 1 and 2; they will presently be described.

In chart 1 graph *A* gives the mitotic count of the original epithelium at some distance from the line of excision as found for normal animals, for those painted with benzene and for those painted with methylcholanthrene. The maximum count obtained after treatment with either substance was about twice the normal, no difference being noticeable between the effects of benzene and methylcholanthrene. The values are, however, slightly higher than those found after painting for one-half or one month.

In graph *B* are given the mitotic counts made at the margin of the excision. In each case the peak of mitoses was seen three days after the making of the wound. Painting of the skin with benzene or methylcholanthrene for two or three months raised the maximum count to seven or seven and one-half times the usual, respectively, compared with six times in untreated skin. During the later stages of repair the mitotic activity declined. Under the influence of benzene this decline was rapid; it progressed more slowly in the methylcholanthrene-treated skin and after ten days was three times the normal, compared with twice the normal in both the nonpainted and the benzene-treated epidermis.

Graph *C* shows the mitotic counts of the new epithelium. As in untreated skin, the number of mitoses rose up to the fifth day after the

excision and then returned to lower or even normal values. The peak of the mitoses (six and a half times normal) seen after treatment with methylcholanthrene was one and a half times higher than that in untreated epidermis (five times the normal) and two and a half times higher

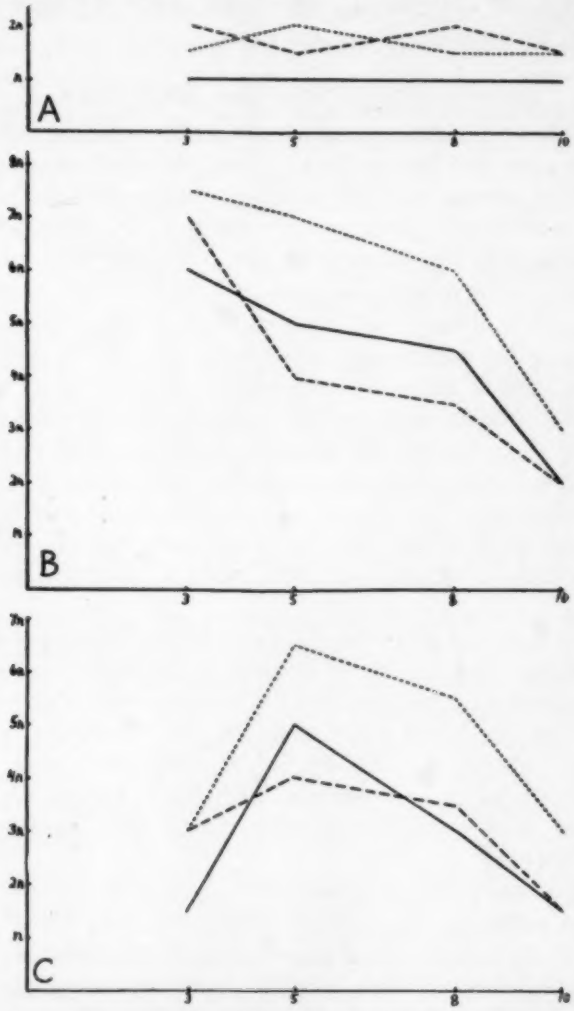


Chart 1.—Mitotic cycles in the epithelium of the ear during ten days of wound-healing of untreated skin (straight line), of skin treated with benzene for two or three months (broken line) and of skin treated with 20-methylcholanthrene for the same periods (dotted line). The mitotic counts are demonstrated in multiples of the normal (*n*) as designated on the ordinate. The number of days of treatment is shown on the abscissa. *A*, epithelium distant from the wound. *B*, marginal epithelium. *C*, new epithelium.

than that seen after application of benzene (four times the normal). Moreover, as in the marginal epithelium, the number of mitoses declined

more slowly than in either the untreated or the benzene-treated skin, and it was still three times the normal ten days after operation, compared with one and a half times the normal in both the latter.

In chart 2 graph *A* illustrates conditions in the epithelium of the flank at a distance from the wound margin. The highest mitotic count obtained was twice the normal, and it occurred after treatment with either

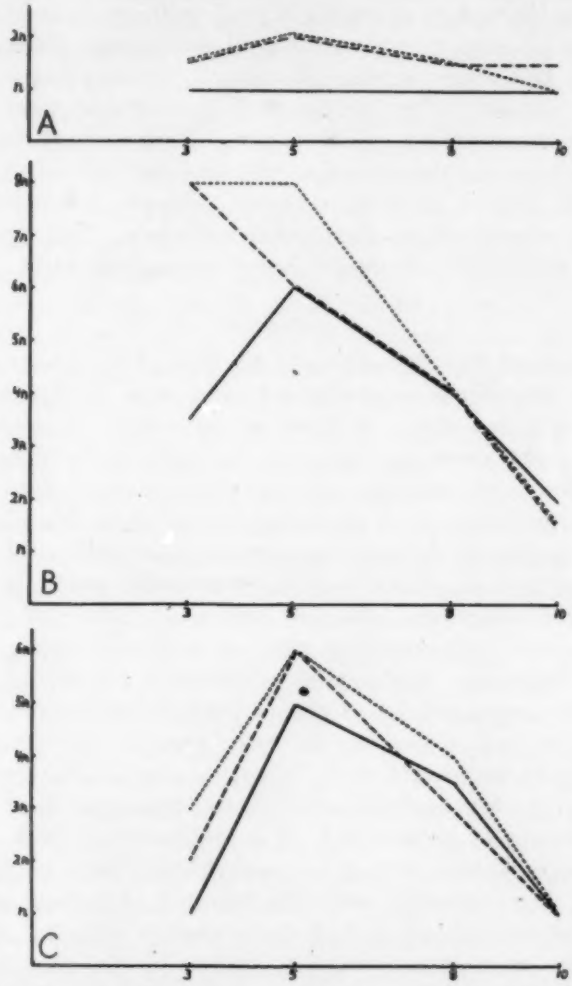


Chart 2.—Mitotic cycles in the epithelium of the flank during ten days of wound-healing of untreated skin (straight line), of skin treated with benzene for two or three months (broken line) and of skin treated with 20-methylcholanthrene for the same periods (dotted line). The mitotic counts are demonstrated in multiples of the normal (*n*) as designated on the ordinate. The number of days of treatment is shown on the abscissa. *A*, epithelium distant from the wound. *B*, marginal epithelium. *C*, new epithelium.

of the two substances. The mitotic counts declined at similar rates during the later stages of healing.

Graph *B* demonstrates the mitotic counts of the epithelium at the margin of the wound of the flank. The maximum of mitoses was present after three or five days of repair. After painting with either benzene or methylcholanthrene the mitotic count reached a high of eight times the normal, whereas in the untreated skin it was six times the normal. During the later stages of healing the fall in the number of mitoses was rapid, and ten days after the making of the wounds a return to values one and a half times or twice the normal had occurred in all groups.

Graph *C* shows the mitotic counts of the new epithelium of the flank. The three curves took a similar course. They reached their peak five days after excision; the maximum was six times the normal after treatment with benzene or with methylcholanthrene, compared with five times the normal in the nonpainted epidermis. Ten days after the operation the number of mitoses had dropped to normal.

COMMENT

In untreated skin of the ear and the flank of the guinea pig, wounds 4 mm. in diameter were epithelized eight days after operation. The measurements and counts obtained in the present experiments are in agreement with those established in the early work of Loeb and his collaborators on wound-healing in the epidermis of this species.[3]

Under the influence of either benzene or methylcholanthrene there were no changes in the time required for epithelization of the defects. There was an insignificant increase of epithelial proliferation at some distance from the wound after one-half or one month of painting, but epithelial growth was intensified after two or three months of application of either substance. The mitotic proliferation was of the same order in the benzene-treated and in the methylcholanthrene-treated skin, but the epidermis was thicker in the latter group. The same conditions were noted at the margin of the excision. Here, also, the increase in mitotic activity was negligible after shorter treatment, but became more obvious after prolonged painting. The proliferation as well as the thickness of the epithelium seen in the methylcholanthrene groups exceeded somewhat those occurring under the influence of benzene alone. Similarly in the new epithelium, both the number of epithelial cell rows and the mitotic count were somewhat higher in the skin treated with methylcholanthrene. Thus there was a slight but definite superiority of methylcholanthrene as compared with the solvent benzene as regards stimulation of epidermal growth. In the connective tissue benzene and particularly methylcholanthrene caused enhanced vascularization, edema and increased formation of collagen, which after prolonged treatment underwent fragmentation.

A comparison of the ear and the flank with respect to wound repair gave the following results: In both areas the defects were closed eight days after excision in spite of the differences in the thickness of the epidermis and the number of mitoses seen normally in these two regions. The epidermis of the ear increased in thickness by about one-fourth after being treated with either benzene or methylcholanthrene; in the flank it was almost doubled in thickness. Under the influence of both painting and the wound, the epithelium of the ear increased two and a half times, whereas an increase of three and a half times the normal was seen in the flank. Thus, under the influence of the carcinogen the thinner epidermis of the flank increased in thickness more markedly than the thicker epidermis of the ear. The maximum number of cell rows seen in both locations was five. This seems to indicate that there are limits to the thickening of the skin. Once a certain number of cell rows has been reached, further increase becomes difficult; instead, the superficial layers undergo keratinization and are sloughed off. On the other hand, the mitoses did not show this limitation. The absolute number of mitoses in a given number of basal cells was higher in the ear than in the flank. Their relative increase occurring under the combined stimulus of the wound and the carcinogen was slightly higher in the ear than in the flank. This difference manifested itself particularly during the later stages of healing, when the mitotic counts returned to normal somewhat more slowly in the ear than in the flank.

As compared with the skin of the mouse, the epidermis of the guinea pig showed only a slight reaction after the application of benzene. Both species responded with intensified proliferation of the epithelium. In the mouse, this stimulation was already noticeable after two weeks of treatment, and it was sufficient to accelerate wound healing. In the guinea pig, a noteworthy stimulation of epithelial growth was seen only after two or three months of painting, but it was not enough to hasten the epithelization of the wounds. A difference in the response of the skin of the two species was present as early as one-half or one month after the start of treatment with methylcholanthrene, but it was more conspicuous after prolonged painting. Epilation was a rare occurrence in guinea pigs, and it affected only small, scattered areas of the epidermis. On the other hand, epilation occurred in most mice and extended all over the painted area. In the latter species, the mitotic count of the original epithelium was increased seven or eight times over the normal, and tumors developed during the period of painting. In the guinea pig, the number of mitoses in the original epithelium did not rise above twice the normal, and it is therefore not surprising that neoplasms did not develop. Moreover, in the healing wounds of guinea pigs the period of intensified mitotic activity was not appreciably prolonged as in mice, nor was there any inhibition of the migration of the epithelium

covering the defect as seen in the latter species under corresponding conditions.[3] Thus the change in the skin of the guinea pig remained restricted to mild hyperplasia, with only slight modifications of the processes of repair, effects partly attributable to the solvent benzene and slightly intensified by methylcholanthrene. In the mouse, by contrast, the true carcinogenic effect of methylcholanthrene manifested itself when this change gradually assumed neoplastic aspects, which coincided with marked disturbances in the epithelization of the wounds.

The reasons for the guinea pig's resistance to carcinogenic substances are unknown. Howes[4] assumed that methylcholanthrene placed into the subcutis of the guinea pig does not destroy the polymorphonuclear leukocytes, which absorb the carcinogen and remove it, whereas in the mouse these cells are destroyed by the carcinogen, which thus can exert its effect on the tissues. However, in the present experiments the methylcholanthrene was applied directly to the skin, where leukocytes do not so readily accumulate as in the subcutis. This makes it probable that the resistance of the guinea pig is based not only on the activity of the leukocytes but also on as yet unknown properties of the epithelium itself. The failure of the hair to fall out under the influence of the carcinogen suggests the possibility that the latter may not be absorbed by the sebaceous glands as it is in the mouse.[5] That the age factor plays any role in the resistance of the guinea pig seems unlikely, since five years' continuous cutaneous application of dibenzanthracene failed to produce tumors.[6]

SUMMARY

Painting of the skin of guinea pigs with 20-methylcholanthrene dissolved in benzene or with benzene for periods of two or three months causes slight hyperplasia of the epithelium. The normally thinner epithelium of the flank responds to the painting with a greater increase in thickness but with relatively no greater mitotic activity than the naturally thicker epithelium of the ear.

Treatment with benzene or methylcholanthrene does not alter the time required for the epithelization of dermal defects in guinea pigs as it does in mice. However, the proliferation of the regenerating epithelium is somewhat stimulated after prolonged treatment with either benzene or methylcholanthrene. Methylcholanthrene exerts a slightly more marked stimulation than benzene.

The greater resistance of the epidermis of the guinea pig as compared with that of the mouse subjected to the influence of carcinogenic hydrocarbons thus manifests itself also in the diminished effect of the latter on wound healing.

4. Howes, E. L.: Cancer Research **6**:298, 1946.
5. Simpson, W. L., and Cramer, W.: Cancer Research **3**:515, 1946.
6. Reimann, S. P.: Am. J. Roentgenol. **43**:275, 1940.

Case Reports

HISTOPLASMOSIS IN INFANCY

The Pathologic Picture as Seen in One Case

LYLE A. WEED, M.D.; ALEXANDER M. IAMS, M.D.*, and
HADDOW M. KEITH, M.D., Rochester, Minn.

SINCE Darling's first article,[1] in 1906, describing the disease which he later called histoplasmosis, 88 cases have been reported in the literature.[2] Twenty-eight of the patients were children. Twenty were infants less than 15 months of age. Because in few of these cases was histoplasmosis diagnosed clinically and because the case which we have observed presents some interesting features, a report with a complete pathologic and bacteriologic description may be of value. A discussion of the clinical features as seen in infancy will be published elsewhere.[3]

REPORT OF CASE

A girl was born in Illinois on April 7, 1945, after a full forty weeks' gestation. The infant weighed 7 pounds 8 ounces (3,402 Gm.) at birth and was in normal health until June 1945, when she was seen by the family physician because of irritability and sleeplessness. She responded well to iron therapy.

In October 1945, at 6 months of age, the patient again was taken to the family physician because of icterus, pallor and a temperature of 103 F. Examination revealed hepatosplenomegaly, rales and decreased breath sounds on the left side of the thorax posteriorly. At this time the concentration of hemoglobin was 7.5 Gm. per hundred cubic centimeters of blood; the erythrocytes numbered 2,760,000 and the leukocytes 3,900 per cubic millimeter of blood, with a normal differential count. The cerebrospinal fluid showed no abnormality. A roentgenogram of the thorax revealed no change from normal. Chemotherapy and antibiotic therapy were instituted without avail.

On November 13 the patient was referred to the Mayo Clinic and was admitted to a hospital for further study and treatment. Physical examination on admission to the hospital showed a well nourished, well developed pale infant with moderate hepatosplenomegaly.

The concentration of hemoglobin was 12.6 Gm. per hundred cubic centimeters of blood; the erythrocytes numbered 3,640,000 and the leukocytes 1,900 per cubic millimeter of blood. Blood smears showed hypochromic, macrocytic anemia, with increased regeneration and occasional basophilic stippling. The concentration of serum bilirubin was in the normal range; the cephalin flocculation reaction was positive; the sulfobromophthalein test showed retention, grade 1 (on the basis of

* Fellow in Pediatrics, Mayo Clinic.
From the Section on Pediatrics, Mayo Clinic.

1. Darling, S. T.: J. A. M. A. **46**:1283, 1906.

2. For a review of the literature see article by A. M. Iams, M. M. Tenen and H. F. Flanagan in the American Journal of Diseases of Children (**70**:229, 1945).

3. Iams, A. M., and Keith, H. M.: Histoplasmosis in Infancy: Report of a Case in an Infant with a Brief Clinicopathologic Review, unpublished data.

1 to 4, in which 1 designates the least and 4 the greatest retention). The concentration of proteins was 5.8 Gm. per hundred cubic centimeters of serum, with an albumin-globulin ratio of 3.06:1. Roentgenograms of the thorax, the head and the long bones were normal.

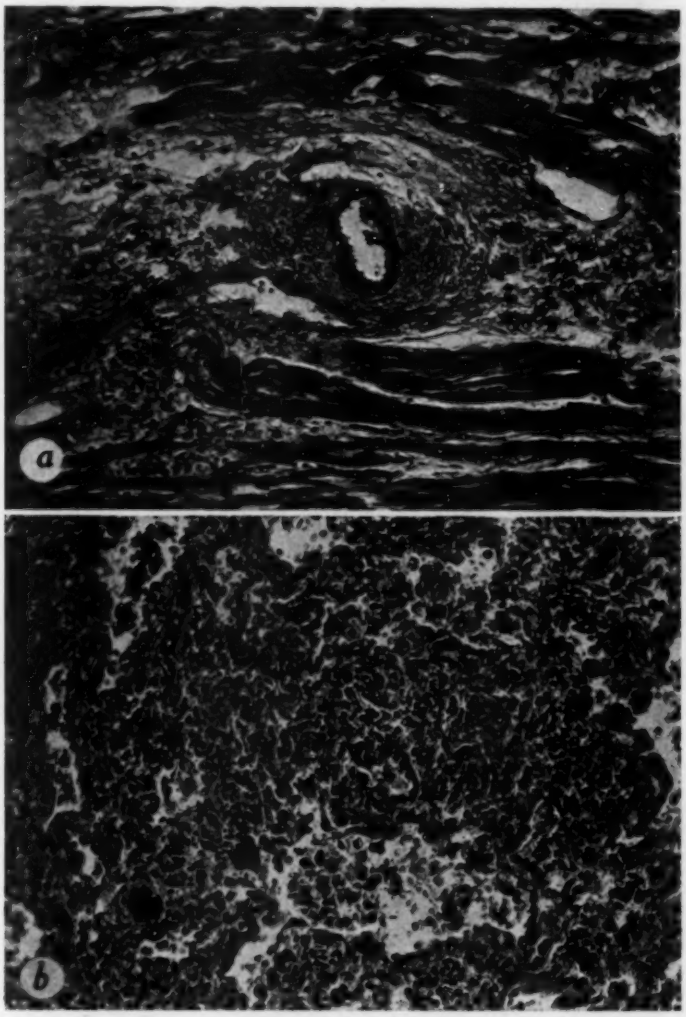


Fig. 1.—(*a*) Heart showing perivascular accumulation of phagocytes, beginning fibroblastic proliferation and destruction of the wall of an arteriole (× 130). (*b*) Lung showing thickened alveolar walls with phagocytes in the alveolar spaces (× 150).

The patient's course was stormy. The rectal temperature ranged daily from 99.6 to 104 F., with peaks at 8 a. m. and 8 p. m. Repeated studies of the blood showed progressive hypochromic macrocytic anemia. Because of the history, the physical findings, the anemia and the leukopenia, histoplasmosis was suspected,

and a biopsy of bone marrow was done on November 23 by Dr. Hargraves, of the Division of Medicine, Mayo Clinic. Typical organisms of Histoplasma capsulatum were found in neutrophils, monocytes, reticuloendothelial cells, eosinophils and megakaryocytes, as well as free in the smear itself.

Dermal tests were made by applying histoplasmin to the infant's forearms in dilutions of 1:1,000, 1:500, 1:100 and 1:10 with proper controls. There were no significant reactions in forty-eight and seventy-two hours. A similar dermal test was made on the forearm of the patient's mother, with a positive reaction in forty-eight hours, the erythematous area measuring 5.4 by 3.6 cm. The erythematous area of the control reaction measured 2.4 by 1.6 cm.

Cultures of blood, sternal marrow, stools and duodenal contents all showed H. capsulatum in five to twenty days. Repeated cultures of urine were negative.

While in the hospital, the patient received penicillin, repeated blood transfusions and Neostam (the nitrogen glucoside of sodium para-aminophenyl-stibonate) without any appreciable effect. Her condition became progressively worse and she died on December 16.

Because of the positive reaction to the histoplasmin dermal test, the patient's mother was examined for signs of latent histoplasmosis. The examination failed to reveal anything abnormal.

Necropsy.—The body was that of an 8 month old white girl measuring 72 cm. in length and weighing an estimated 20 pounds (9.07 Kg.). There were numerous petechiae scattered over the abdomen and the thorax.

The heart weighed 55 Gm. The epicardium, the endocardium, the valve leaflets and the myocardium appeared grossly normal. Sections for histologic examination showed diffuse interstitial proliferation of fibroblasts and macrophages with a moderate number of infiltrating lymphocytes and plasma cells. There was a tendency for such regions of reaction to be more prominent in the vicinity of the blood vessels. The macrophages contained varying numbers of spherical bodies characteristic of the yeast form of H. capsulatum. Although the reaction was primarily between the muscle fibers, there were many regions in which the fibers were abruptly interrupted. The cross striations and the muscle nuclei appeared normal right up to the frayed ends, suggesting that the fibers had been destroyed in the process (fig. 1 *a*).

The visceral pleura of the lungs contained many petechiae. The lungs were pale pinkish red and firm with an increased consistency resembling organizing pneumonia. They were so firm that after removal from the thorax they retained their original size and shape. The palpable air-containing tissue was confined to the anterior borders adjacent to the pericardium. The cut surfaces were uniformly pale grayish pink, and from them no demonstrable exudate could be scraped. The regional lymph nodes were enlarged, discrete, firm, pale pink and moist on the cut surface. Histologically, the lungs showed extensive proliferation of fibroblasts, with thickening of the alveolar walls, producing diffuse organizing pneumonia. The over-all reaction was that of diffuse proliferation with a minimal amount of exudate (fig. 1 *b*), and occasional local regions somewhat suggestive of tubercles were found. Indeed, the proliferative reaction was so prominent that it was usually difficult to find the etiologic agent in the routine sections stained with hematoxylin and eosin, although cultures proved the organisms to be present in large numbers.

The liver weighed 556 Gm. and was light brown mottled with yellowish brown areas approximately 1.0 cm. in diameter. The anterior border was rounded. The capsule was smooth. The organ was uniformly firm and the consistency

moderately increased. The cut surface was relatively dry and reddish brown mottled with yellowish brown areas ranging from 1.0 to 2.0 cm. in diameter. Histologically, there were foci of necrosis showing macrophages filled with organisms and extensive fibroblastic proliferation without significant leukocytic infiltration. The periportal regions showed huge numbers of macrophages engorged with yeast forms. In these regions early fibrosis was extensive. The Kupffer cells were laden with organisms and a finely granular material. Many blood vessels were filled with macrophages distended with phagocytosed yeastlike bodies. In many regions these masses appeared to form emboli. There were regions of necrosis in the walls of some of these vessels (fig. 2).

The spleen weighed 306 Gm. and was firm and rubbery. The surface was covered with a mild fibrinous exudate. The cut surface was purplish red, and large amounts of pulp of a similar color could be scraped from it. The follicles were prominent, large and well demarcated. Histologically, there were numerous

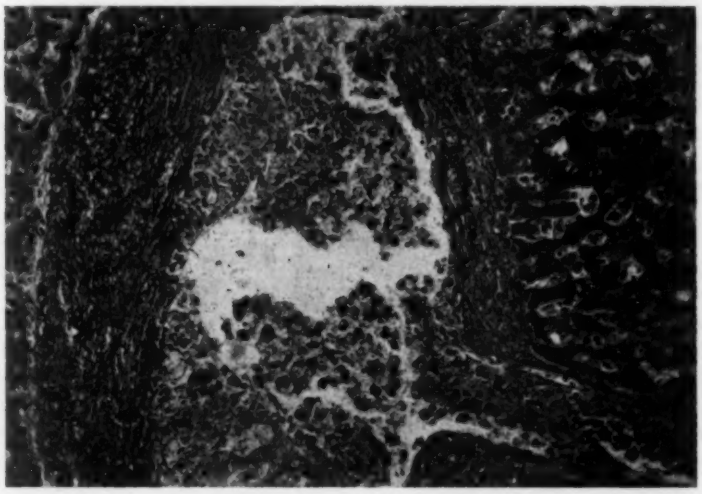


Fig. 2.—Liver showing necrosis of vessel walls with the lumen filled with yeast-laden phagocytes. Yeasts englobed by the Kupffer cells are less distinct at this magnification (× 100).

regions of necrosis containing nuclear dust surrounded by numerous macrophages distended with the yeast forms of the organism. Intermingled with the phagocytes were numerous fibroblasts. Between the necrotic regions the splenic structure was obliterated with extensive fibroblastic proliferation and yeast-laden phagocytes. Impression smears from the spleen stained by Wright's and Giemsa's technics showed large numbers of the organisms within the phagocytes.

The right and left kidneys weighed 54 and 57 Gm., respectively. The cut surfaces were pale pink, and the cortical striations were scarcely visible. Scattered throughout the cortices were a few small, light yellowish brown regions suggestive of tubercles. The medullary portions were somewhat darker, and the radial striations were clearly visible, thus sharply demarcating these portions from the cortices. There were no grossly visible abnormalities of the papillae, calices, pelves or ureters. Histologically, the kidneys showed scattered foci of fibroblastic proliferation, especially in the cortices, although these foci were present throughout

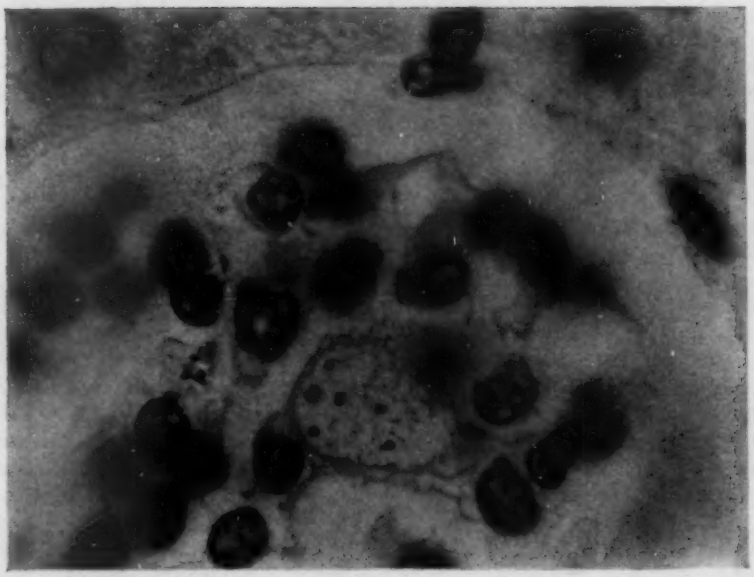


Fig. 3.—Glomerulus showing Histoplasma capsulatum in the capillary loops (× 1,350).

the organs. Such regions contained yeast-laden phagocytes and fibroblasts, with minimal leukocytic infiltration. The capsules of Bowman contained some albumin. There was evidence of diffuse proliferation of the glomerular tufts, and in many of the glomeruli, organisms of Histoplasma could be seen in groups of three or four (fig. 3). The tubules showed mild granular degeneration. In the mucosa of the renal pelvis were many phagocytes filled with the yeast forms of Histoplasma.

The right adrenal gland appeared grossly normal, but there were small regions resembling miliary granuloma in the medulla of the left adrenal gland. The dark cortical portions were sharply demarcated from the medullary portions. The medulla had the appearance of being increased in amount and in some regions extended to the surface by replacing the cortex. There were no grossly visible localized regions of necrosis or hemorrhage. Histologically, the medullae were replaced with macrophages filled with yeastlike forms. There was extensive destruction of the fascicular layer, with the organisms actually existing in the cortical cells. The reaction appeared to be that of a progressive disintegration extending from the medulla outward by contiguous invasion of the cells of the cortical fasciculi (fig. 4*a* and *b*). In some places this invasion had extended entirely through the cortical layer to the surface of the gland. There was only mild fibroblastic proliferation in the adrenal glands in contrast with that seen in the other organs.

There were no grossly visible lesions in the esophagus, the stomach, the small or the large intestine. However, histologic examination of the esophagus showed focal accumulations of yeast-laden phagocytes with an associated fibroblastic proliferation beneath the mucosa (fig. 5*a*). The reaction suggested that, if the patient had lived longer, ulceration would have taken place eventually. It also suggested that the ulcer formation cited by other investigators may be due to breaking down of such foci resulting from hematogenous dissemination and not to erosion of the surface from without. In the large bowel there were regions in which the lamina propria was distended with phagocytes containing enormous numbers of Histoplasma (fig. 5*b*). In these regions there was desquamation of the epithelium, forming microscopic ulcers. In many regions the organisms were present in clusters of phagocytes beneath the serosa and between the muscular layers.

The urinary bladder was grossly normal. Microscopically, there were collections of phagocytes only in the outer portion of the wall in the connective tissue. The urine was slightly cloudy but did not have the gross appearance of pus. Microscopic examination showed it to contain phagocytes filled with yeastlike organisms.

There was generalized enlargement of the lymph nodes, which remained discrete. The nodes from the periaortic, pelvic, mesenteric, peripancreatic, cervical, axillary and inguinal regions were similar grossly and microscopically. Histologically, they showed regions of necrosis and fibroblastic proliferation with large numbers of phagocytes filled with Histoplasma. Impression smears taken from them and stained by the Wright and Giemsa technics showed enormous numbers of organisms within the phagocytes. There were also many extracellular organisms diffusely scattered throughout the nodes.

The thymus was nodular and pale pink. There were no grossly visible lesions present, and there did not appear to be a significant increase of resistance. Microscopically, there was extensive proliferation of fibroblasts with a few regions of necrosis containing nuclear dust like that seen in caseous tuberculosis. The periphery of such necrotic regions showed fibroblastic proliferation without leukocytic infiltration. Diffusely scattered throughout the tissue were enormous numbers of macrophages engorged with organisms in the yeast form (fig. 6).

A proliferative reaction in tissue adjacent to one parathyroid gland extended into the gland (fig. 7). In the remaining three parathyroid glands there was no microscopic evidence of invasion by, or reaction to, the organisms.

Marrow from the ribs and the vertebral bodies was reddish brown and grossly appeared normal. The impression smears stained by the Wright and Giemsa technics showed enormous numbers of organisms in the phagocytes.

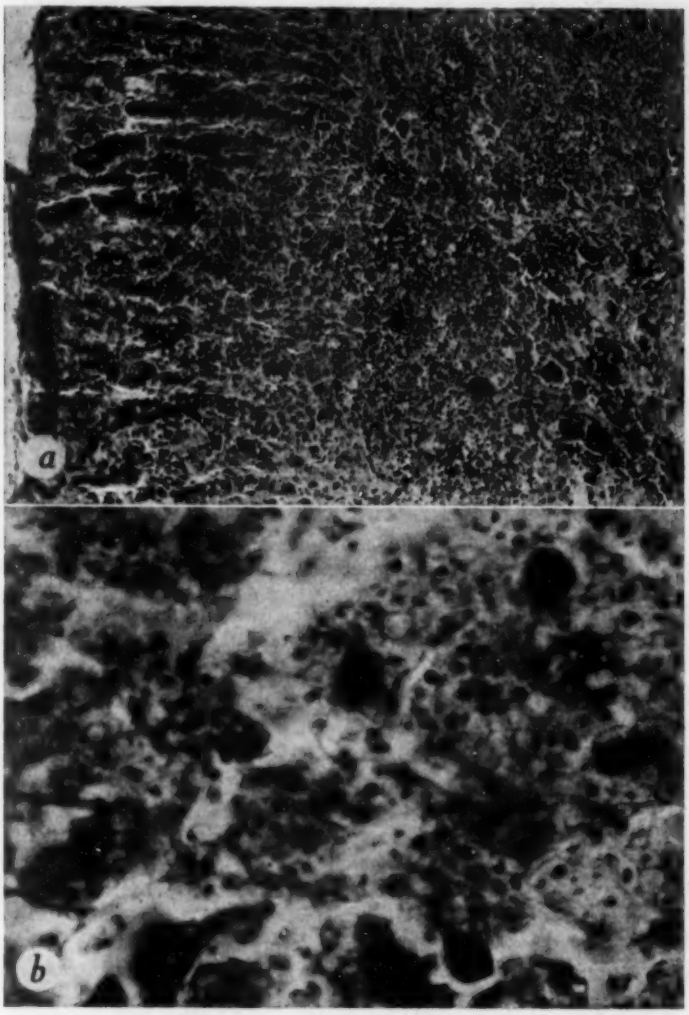


Fig. 4.—(*a*) Adrenal gland showing replacement of the medulla with myriads of yeast forms in phagocytes, the process extending outward into the cortical fasciculi (× 100). (*b*) Cortical cells of (*a*). Note the distention with large numbers of organisms and the obliteration of architecture (× 1,350).

There were no gross or microscopic lesions in the dura, the pia-arachnoid, the cerebrum, the cerebellum, the spinal cord or the pituitary gland. However, a

section of the spinal cord showed organisms within phagocytes in the blood vessels of the meninges, immediately adjacent to the cord. This would be expected, however, in view of the presence of organisms circulating in the blood as demonstrated by bacteriologic technic. The middle ears were normal.

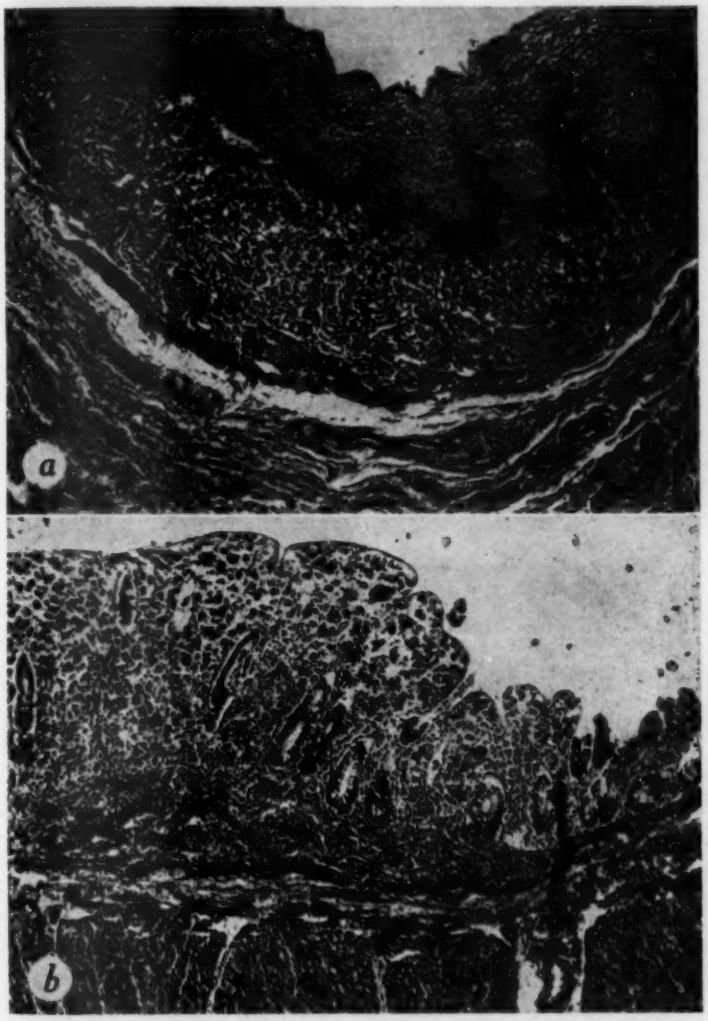


Fig. 5.—(*a*) Esophagus showing accumulation of yeast-filled phagocytes and fibroblastic proliferation in the submucosa (×75). (*b*) Large bowel, showing numerous yeast-filled phagocytes distending the lamina propria (× 50).

The diaphragm was grossly normal, but microscopic examination revealed numerous foci of accumulations of yeast-laden phagocytes intermingled with fibroblasts (fig. 8*a*).

Bacteriologic Examination.—The following materials were subjected to bacteriologic examination in an attempt to isolate the organism H. capsulatum: blood, lung, liver, spleen, kidney, rectus abdominis muscle, lymph nodes, frontal lobe of

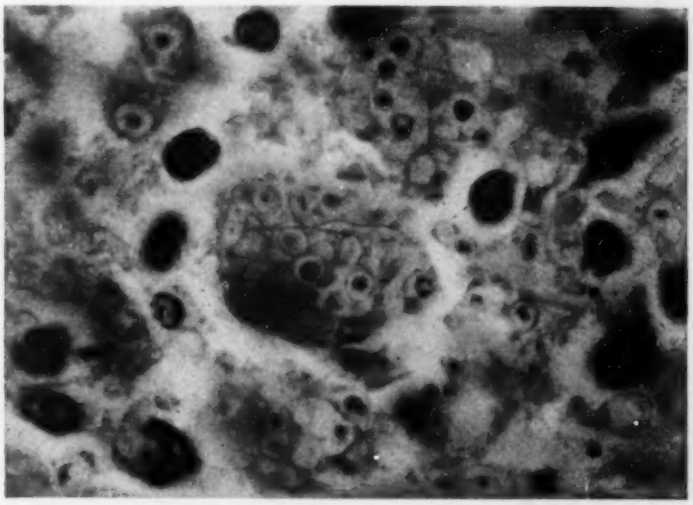


Fig. 6.—Thymus showing organisms in the phagocytes (× 1,350).

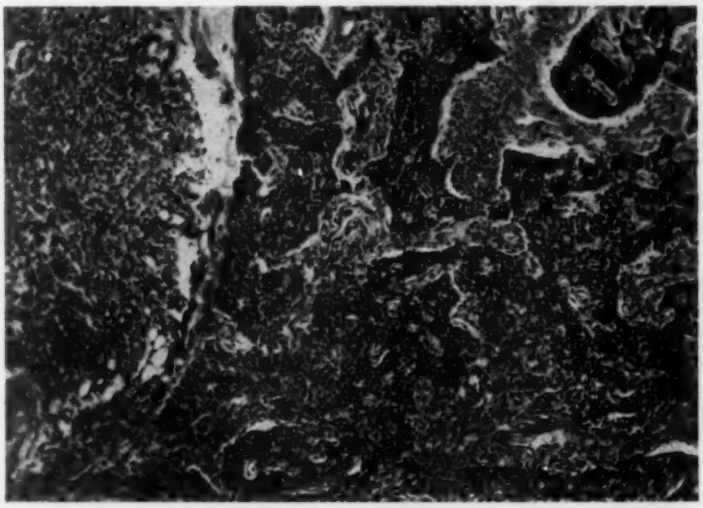


Fig. 7.—Parathyroid gland. The fibroblastic proliferation at the left is extending into the gland as dense cords (× 85).

brain, marrow from a vertebral body, cerebrospinal fluid, bile, urine and stool. Blood was drawn aseptically from the heart after the skin over the precordium had been cauterized. Cerebrospinal fluid was removed from the cisterna magna in a similar fashion. Likewise a sample of urine was obtained from the urinary

bladder and bile from the gallbladder. Marrow was obtained from the vertebral bodies by squeezing them and removing the extruded material. Specimens of lung, liver, spleen, kidney, rectus abdominis muscle, lymph nodes and frontal lobe of the brain were removed aseptically by cauterizing the surface of the respective organ and excising the tissue with sterile scissors. These instruments were submitted to dry heat at 170 C. for two hours to eliminate the theoretic possibility that organisms might be carried over on them, as might have been the case had they been prepared by boiling.

Blood, cerebrospinal fluid, urine and bile were cultured directly. The tissues and the stool specimen examined were emulsified in saline solution, and the

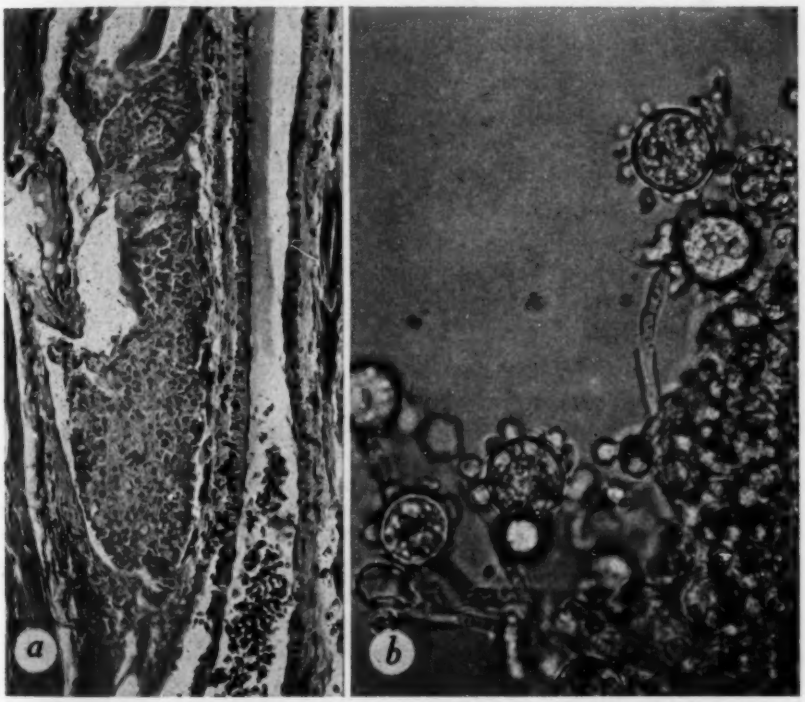


Fig. 8.—(*a*) Diaphragm showing numerous phagocytes filled with organisms (× 105). (*b*) Tuberculate chlamydospores produced by the organism isolated from the patient (× 900).

emulsions were used as inoculums. The materials were cultured on 7 per cent horse blood agar containing 20 units per cubic centimeter each of penicillin and streptomycin, and the plates were incubated at 30 to 32 C. and at 38 C. Portions of blood, cerebrospinal fluid, bile, urine and emulsions of spleen, liver and lung were also inoculated in 7 per cent horse blood broth at 38 C. in an attempt to produce cultures in the yeast form.

Growth was more rapid in plates incubated at 30 to 32 C. than in those incubated at 38 C. No yeast forms were produced at the higher temperature, although filamentous forms were numerous. At 32 C. numerous tiny white microaerophilic filamentous colonies were present after four to five days, These gradually increased

in size, ranging from 5.0 to 20.0 mm. in diameter, depending on the crowding of the colonies. When the inoculum was heavy—for example, those from the spleen, lymph nodes or marrow—the adjoining colonies fused to form a matlike growth. The early growth was composed only of branching filaments radiating from a central tangled mass. Colonies seven to ten days old contained well developed chlamydospores. In colonies two to three weeks of age the diagnostic tuberculate chlamydospores developed as seen in figure 8*b*. The organisms were isolated from specimens of blood, lung, liver, spleen, kidney, marrow, rectus abdominis muscle, frontal lobe of the brain, urine and stool. They were not isolated from cerebrospinal fluid or bile. The absence of the organisms from the cerebrospinal fluid and the lack of histologic evidence of involvement of the brain suggest that the cultural demonstration of organisms in the frontal lobe was probably due to their presence in the blood vessels supplying the tissue examined. After the material had been kept for six weeks in the icebox, the organisms were still viable and retained their original characteristics.

COMMENT AND SUMMARY

The evidence obtained from a study of the pathologic material of this case indicates widespread dissemination of the Histoplasma. The finding of the organisms in the blood, the marrow and lymph nodes suggests that every organ and tissue was involved to some degree, although on microscopic examination some specimens showed no fixed tissue reaction to the presence of the organisms. Whether this represents variation in tissue immunity or some other mechanism cannot be determined from the information at hand. The condition of the adrenal glands is especially interesting in this connection. The yeast forms appeared to be actually invading the cortical cells, some of the latter containing so many parasites that they lost their identifying characteristics and appeared as macrophages. To consider either the cortical cells as phagocytes or the yeast forms as invaders is unique since in no other condition has the former been demonstrated and in other parts of the body the organisms occurred only in phagocytic cells.

CONGENITAL TULAREMIA

THOMAS N. LIDE, M.D., DURHAM, N. C.

REPORTS of tularemia occurring during pregnancy are not numerous and are most often included in general considerations of the disease. None I have been able to find mentions either tularemic interruption of pregnancy or tularemia occurring in the fetus. Bowe and Wakeman [1] recorded a case in which the mother acquired tularemia during midpregnancy, recovered and delivered a normal infant whose blood agglutinated Pasteurella tularensis in dilutions up to 1:80 at the time of birth. Three other patients are mentioned by Kavanaugh.[2] All went to term and delivered normal infants. Pullen and Stuart [3] likewise mentioned 3 patients who acquired the disease during pregnancy and later delivered apparently normal infants.

The present case of fetal infection with intrauterine death, and recovery of the mother is thought to be the first reported.

REPORT OF A CASE

A 30 year old white married woman, octipara and in the eighth month of her ninth pregnancy, was admitted to Duke Hospital on Dec. 3, 1945, complaining of a "sore" on the left third finger of about two weeks' duration. She had prepared a rabbit, caught by a dog, one week before the onset of her illness, and had subsequently during that week dressed or prepared two other rabbits for the table. A small ulcer appeared on the left third finger, associated with enlargement and pain of the epitrochlear and axillary lymph nodes and general aching. One week after the onset, nausea and vomiting developed after meals, and she noted that her temperature was elevated, with daily fluctuations rising as high as 39.4 C. (102.9 F.). She had no chills. More exact relationships of exposure and development of symptoms were not remembered.

Her last menstrual period was in April 1945, and the expected date of confinement was in January 1946.

It is of interest that two sons of the patient, aged 14 and 8 years, were confined to this hospital at the same time as the mother. Both had tularemia. In the older boy the disease was ulceroglandular, while in the younger it was of the oculoglandular type. Both recovered.

Physical Examination.—The patient's temperature was 37.5 C. (99.5 F.); pulse rate, 100; respirations, 18. The patient appeared chronically ill, but was a well developed and well nourished white woman. There was an ulcer 1.5 cm. in diameter with a necrotic base on her left third finger. The left epitrochlear and axillary lymph nodes were enlarged and tender. No other cutaneous or lymphatic

---

From the Department of Pathology, Duke University School of Medicine.

1. Bowe, D. P., and Wakeman, D. C.: J. A. M. A. **107**:577, 1936.
2. Kavanaugh, C. N.: Arch. Int. Med. **55**:61, 1935
3. Pullen, R. L., and Stuart, B. M.: J. A. M. A. **129**:495, 1945.

lesions were noted. The heart and the lungs were clear. The fundus of the uterus was palpable 2 cm. above the umbilicus; fetal movements were active, and fetal heart sounds were heard best in the right lower quadrant of the abdomen.

Accessory Clinical Findings.—At the time of admission the leukocyte count was 7,800 per cubic millimeter, with a differential count of 58 per cent segmented, 15 per cent stab and 5 per cent juvenile neutrophilic polymorphonuclear leukocytes and 22 per cent lymphocytes. The corrected sedimentation rate was 36 mm. per hour. An uncatheterized specimen of the urine contained 3 to 10 red blood cells per high power field but was otherwise normal. Agglutination of P. tularensis occurred in dilutions of 1:2,560 on the day of admission.

Course in Hospital.—The patient was afebrile for the first seven days of her hospital stay. The ulcer on the finger healed well under treatment with saline compresses. Agglutination tests for P. tularensis on the fourth and eighth days of admission resulted in the same agglutination titer as did that of the day of admission. On the sixth day the patient stated that she felt no fetal movements, but the fetal heart sounds were still audible in the right lower quadrant of the abdomen. On the eighth day her temperature rose to 38.6 C. (101.4 F.) and on the ninth day to 40.5 C. (104.9 F.). Her leukocyte count had meanwhile risen to 15,200 per cubic millimeter, with a differential count of 51 per cent segmented, 39 per cent stab and 5 per cent juvenile neutrophilic polymorphonuclear leukocytes and 5 per cent lymphocytes. Fetal heart sounds were no longer heard. On the tenth day the patient began to discharge a bloody material per vaginam. On the eleventh hospital day she delivered a macerated male fetus, after which her temperature fell to normal, with subsequent daily fluctuations no higher than 37.8 C. (100 F.). The leukocyte count also fell to normal and remained between 5,200 and 8,900 per cubic millimeter, with normal differential counts. Subsequently her illness was complicated by jaundice, hepatomegaly and splenomegaly, which began on the third postpartum day and cleared rapidly, so that she was discharged to her home on the twenty-second hospital day (eleven days post partum).

Blood taken from the umbilical cord at the time of delivery of the fetus agglutinated P. tularensis in dilutions of 1:2,560.

Autopsy of the Fetus (two days after delivery and approximately five days after death).—The macerated male infant was well formed, measuring 46 cm. in crown-heel length and weighing 2.4 Kg. Sanguineous fluid was found in all the body cavities, and there was gross evidence of postmortem degeneration of all organs.

The heart weighed 19 Gm. and showed no abnormalities. The lungs showed no gross lesions. The spleen weighed 16 Gm. It was firm, enlarged and dark red. The pulp bulged slightly above the cut surface of the capsule. The liver weighed 107 Gm. (normal for this age, approximately 70 Gm.). It was dark red without evidence of localized inflammatory changes. The adrenal glands showed no gross lesions. The weight of the kidneys combined was 23 Gm. These organs were darker than usual and showed only slight fetal lobulations. The thymus weighed 11 Gm. and showed no gross lesions. The brain was so badly autolyzed that it could not be studied. The placenta measured 17 by 12 by 5 cm. and weighed 450 Gm. No gross lesions were noted.

Microscopic Examination.—Throughout the placenta were many granulomatous foci, located predominantly in the intervillous spaces. These foci were composed of round cells, both lymphocytes and mononuclear phagocytes, and a few polymorphonuclear leukocytes enmeshed in deposits of fibrin. Karyorrhectic necrosis

at the center of these lesions was a prominent feature. Many lesions were confluent and involved adjacent chorionic villi, which themselves contained many interstitial mononuclear cells, with necrosis in some of the villi. It appeared that the cells from the fetal circulation were taking an active part in the inflammatory reaction.

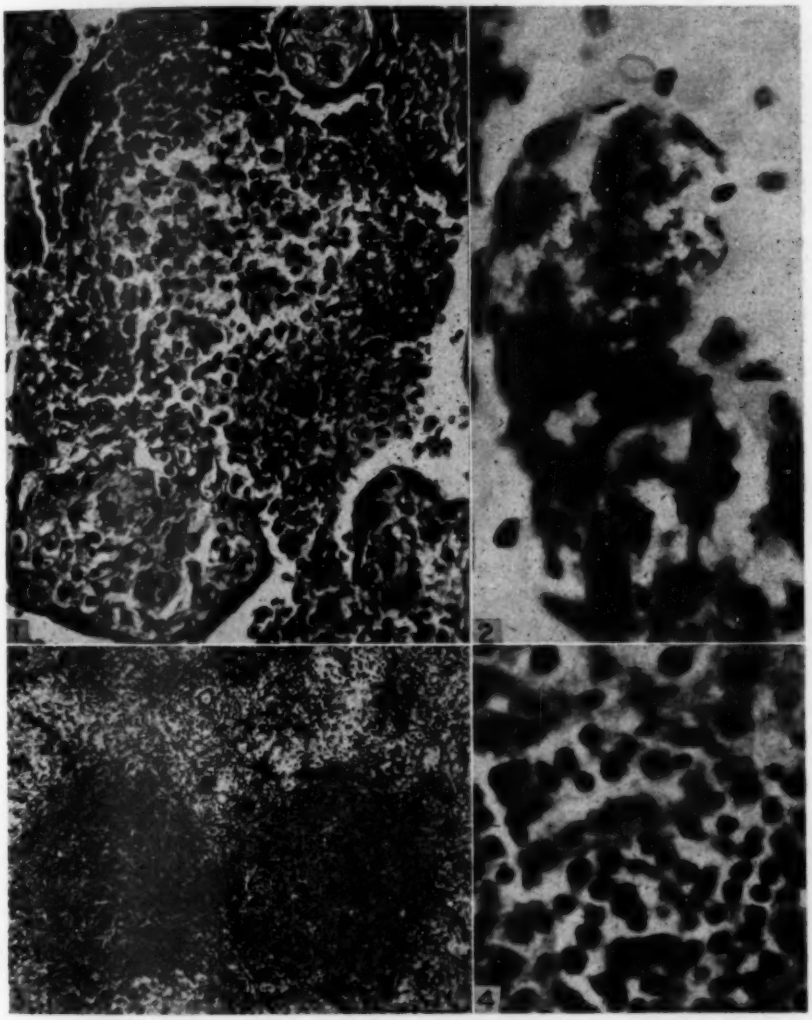


Fig. 1.—Lesion of the placenta. It is characteristic of those found throughout the placenta and shows that chorionic villi were involved in the inflammatory process. × 295.5.

Fig. 2.—Two chorionic villi containing clumps of gram-negative coccobacilli. × 1,478.

Fig. 3.—Lesions of the spleen were similar to those found in the liver, the kidney, the lungs and the marrow. The borders of two additional lesions are visible at the upper margin of the photograph. × 73.9.

Fig. 4.—The mononuclear reaction at the border of the necrotic focus in the capsule of the thymus. × 887.

No lesions were found in the heart.

The capsule of the thymus contained one well preserved lesion, the center of which showed considerable necrosis with surrounding infiltration of round cells and deposition of fibrin. No lesions were noted in the gland itself, which was much better preserved than other tissues.

One small necrotic focus similar to the other lesions was found in the lungs. The tissue was poorly preserved.

The spleen and the liver, despite the marked autolytic changes, showed large numbers of small necrotic granulomas. They were marked particularly by deposits of fibrin with central necrosis and with peripheral infiltration of macrophages and lymphocytes. Many small lesions were confluent.

Similar foci were found in the adrenal glands, in the cortex of each kidney and in the costal marrow.

In sections of the placenta, stained for bacteria by the Brown-Brenn [4] technic, masses of tiny gram-negative coccobacilli were found within two of the villi. The organisms were consistent morphologically with P. tularensis. They are shown in figure 2. Other small gram-negative bodies, apparently the organism, were found also within the macrophages in the placenta and spleen, but no other easily identified masses of bacteria were found.

COMMENT

It is difficult to state with any degree of certainty the time at which the fetus became infected, but evidence suggests that the infection was active in the infant during the fourth week of the mother's illness, when on her sixth hospital day fetal movements became less notable, with fetal death occurring on the ninth day. The mother had been hospitalized on about the twentieth day of her illness. The rise of temperature and of leukocyte count which occurred on the eighth hospital day may well represent her reinfection by the fetus. This suggestion is supported by the fact that the organisms were found within chorionic villi, with placental necrosis, and by the direct communication between maternal and fetal circulations. The fact that the temperature and the leukocyte count rapidly returned to normal after delivery also supports the suggestion of reciprocal infection.

The finding of the organisms in the tissues is also unusual. Lillie and others [5] have pointed out the difficulty of identifying P. tularensis in human material, and in only 2 of the 26 cases which they reviewed was the organism found in the tissues. In 3 cases reported by Thomas [6] organisms consistent morphologically with P. tularensis were found in smears of material taken from various organs at autopsy. Matthews [7] (cited by Ashburn and Miller) reported finding the organisms in tissues taken at autopsy in 1 case. Ashburn and Miller [8] also found intra-

4. Brown, J. H., and Brenn, L.: Bull. Johns Hopkins Hosp. **48**:69, 1931.

5. Lillie, R. D., and others: The Pathology of Tularaemia, National Institute of Health Bulletin 167, United States Treasury Department, Public Health Service, 1936, pp. 73-75.

6. Thomas, H. B.: Ann. Int. Med. **17**:659, 1942.

7. Matthews, W. R.: New Orleans M. & S. J. **90**:479, 1938.

8. Ashburn, L. L., and Miller, S. E.: Arch. Path. **39**:388, 1945.

cellular organisms, which they identified as P. tularensis, within macrophages in the lung of a patient who died during the fifth day of her illness.

SUMMARY

A case of congenital tularemia, presumably the first, is presented. The disease occurred in the mother during the eighth month of pregnancy and was followed by infection and death of the fetus. The necrotic granulomas occurring in various organs of the fetus were similar to those which are usually found in adults. Gram-negative coccobacilli consistent morphologically with P. tularensis were demonstrated in chorionic villi.

AINHUM

Report of a Case in Which the Patient Was a White Woman with Diabetes Mellitus

L. J. ORDIE SHAFFER, M.D., MINNEAPOLIS

AINHUM (dactylolysis spontanea) has been differently considered by various authorities as being either a distinct pathologic entity or a symptomatic manifestation of any one of various diseases. The condition is characterized by a gradually constricting fibrous ring most commonly in the digitoplantar fold of the little toe, which deepens and eventually results in a spontaneous amputation. The condition is most commonly found in male Negroes and is more prevalent in Africa, South and Central America and the West Indies but variations in the incidence as regards sex, race and toe involved occur, and 51 cases have been reported in the United States. Clark first noted this condition among the natives of the African Gold Coast in 1860 and reported it under the name of "dry gangrene of the little toe." In 1867 da Silva Lima [1] gave the first adequate description of the condition. Hornaday [2] published the first report of a case in the United States in 1881. Since that time arguments have been advanced for and against the view that ainhum is a distinct disease and although the majority favor the idea that it is an entity, there is evidence supporting the other view. The present report of a case seems timely as it gives added weight to the view that ainhum does occur as a manifestation of various local and general pathologic conditions.

REPORT OF CASE

A white woman aged 57 was known to have been diabetic for six years and had taken 20 units of crystalline insulin daily during the year 1945. She first noticed a corn on the lateral side of the left third toe in July 1944. A minimal infection of the skin developed in the area and continued for two months. At the end of this time all infection had subsided, but a constricting band was present around the toe. The constriction continued to progress during the next sixteen months until the globular toe was attached only by a narrow pedicle. No color changes or further infection occurred in the third toe during the interventing sixteen months. During October 1945 a corn developed on the left fourth toe, and a similar constriction on the lateral and inferior surface of the toe occurred during the following two months. The patient had noted cramps in her legs on walking since August 1945, indicative of intermittent claudication. In December 1944 she bumped her left great toe and a mild infection of the skin occurred in the abrasion. This healed shortly thereafter, but in July 1945 an infection recurred in the great toe, and small amounts of purulent material drained from

From the Department of Surgery, University of Minnesota.

1. da Silva Lima, J. F.: Arch. Dermat. **6**:367, 1880.
2. Hornaday, E. H.: North Carolina M. J. **8**:116, 1881.

the area periodically from that time on. In October 1945 the great toe became painful, and the following month a black discoloration of the medial portion of the great toe occurred. The history of the patient revealed that she was born in Canada of Irish descent but had lived in Minnesota during the last thirty-five years. She had had none of the usual childhood diseases except measles. At 31 years of age a subtotal hysterectomy had been done for uterine myoma. She said that she had never had a venereal disease. One sister also had diabetes, but no other member of the family had any similar disturbance of the feet. On admission to the University Hospital, December 31, she was a moderately obese, well developed white woman in no acute distress. There was diabetic retinopathy with increased tortuosity of the vessels and scattered "cotton wool" exudates. No hemorrhages or papilledema were present however. The mouth was edentulous but otherwise normal. The blood pressure was 140 systolic and 80 diastolic. Neck, heart, lungs

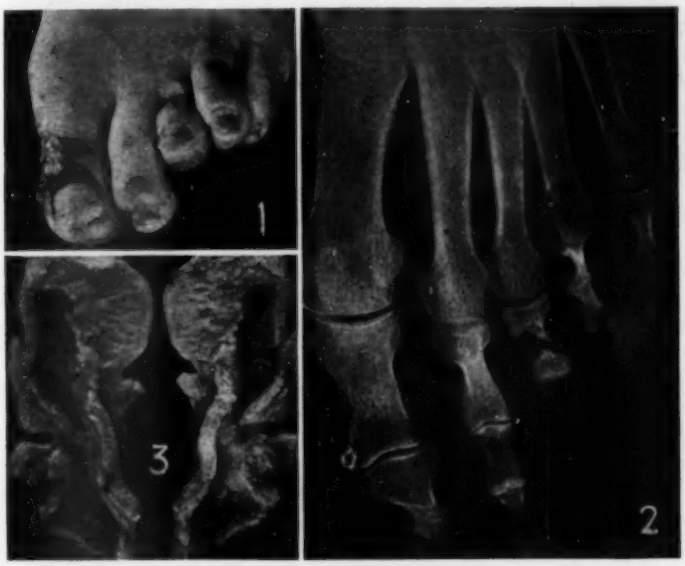


Fig. 1.—Photograph of the left foot showing ainhum of the third toe, beginning constriction of the fourth toe and local areas of superficial dry gangrene on the dorsum of the hallux and the lateral side of the foot.

Fig. 2.—Roentgenogram of the left foot revealing complete severance of the third proximal phalanx and beginning atrophic changes in the fourth proximal and middle phalanges.

Fig. 3.—Medial and lateral halves of the longitudinally sectioned third left toe involved by ainhum. Note the fibrous band severing the proximal phalanx.

and abdomen were all normal except for the presence of a small midline infra-umbilical incisional hernia. There was no perceptible arterial pulsation in either foot, but the right foot was otherwise normal. A deep circular constriction was present around the midportion of the proximal phalanx of the left third toe, which had almost severed the toe. No signs of local inflammation or tenderness were present, however, and the toe could be moved in any direction much as a grape on a stem. A corn was present on the lateral dorsum of the fourth left toe with a constriction on the lateral and inferior surfaces. Areas of dry gangrene without infection were present on a 2 by 5 cm. surface of the medial hallux and on a

1 by 1 cm. area on the lateral surface of the foot at the distal end of the fifth metatarsal. No swelling or erythema of the foot was present (fig. 1). The urine had a specific gravity of 1.025; the hydrogen ion concentration was 5; there was a trace of albumin, sugar (1 plus) and acetone (1 plus); there was no diacetic acid; there were no casts or erythrocytes; the leukocyte content was 3 plus. The hemoglobin value was 12.7 Gm.; the blood leukocyte count was 8,300, with neutrophils 79 per cent and lymphocytes 21 per cent. The sedimentation rate was 91 mm. in one hour (Westergren method). The Kline and Kahn tests were negative. The blood sugar was determined to be 181 mg. per hundred cubic centimeters; the carbon dioxide-combining power, 44 volumes per cent; the blood urea nitrogen, 8 mg. per hundred cubic centimeters and the plasma proteins, 6.4 Gm. per hundred cubic centimeters. Culture of the urine revealed Aerobacter, and culture of the surface of the area of dry gangrene revealed only coagulase-negative staphylococci, nonhemolytic streptococci and diphtheroids present.

Roentgen examination of the left foot revealed a constriction of the soft tissue through the middle of the proximal phalanx of the third left toe with rarefaction and complete severance through the center. Atrophic changes were present in the third middle phalanx, and only a very small portion of the third distal phalanx was visible. Atrophic changes were also visible in the proximal and middle phalanges and the intervening joint space of the fourth toe (fig. 2). The changes were compatible with either ainhum or leprosy, but diagnosis of the latter was not favored, since there were no other bacteriologic or physical findings to substantiate it.

The patient was given parenteral penicillin therapy together with foot soaks for eleven days in the hope that these measures might aid in the demarcation and slough of the superficial dry gangrene. However, no improvement occurred. The first four toes were then amputated and a primary closure done in each case. Unfortunately, as was suspected, although no infection occurred, healing was impaired because of vascular disease. Three weeks were given to offer every possible chance of healing, and then, after McClure-Aldrich tests had revealed impairment below the knee, and histamine flare tests an impairment below the middle of the lower leg, a supracondylar amputation was done on the left leg. The stump healed by primary intention, and the patient was discharged from the hospital on the tenth postoperative day, Feb. 18, 1946. She has had no further difficulty in the short interval since that time.

Pathologic Examination.—Gross examination of a longitudinal midsection of the third left toe revealed a fibrous constriction that had completely severed the bone (fig. 3). A beginning constriction was present on the lateral and inferior surface of the fourth toe. The second toe was normal, but the great toe showed an area of dry gangrene 2.5 by 5 cm. in size on the medial surface. Microscopic examination of the tissue of the third toe revealed the epidermis to be hypertrophied at the point of constriction and just distal to this area. The stratum corneum was moderately thickened. A poorly defined stratum lucidum was present, but the stratum granulosum was normally developed. The stratum malpighii (stratum germinativum) was markedly thickened with elongated, enlarged dermal papillae present, and the plexiform rete cones freely anastomosed with one another. The normal nuclear structure was present in both the stratum granulosum and the stratum malpighii. The reticular layer of the dermis was composed of bundles of collagenous fibers, but a moderate increase in the amount of elastic fibers was present throughout the dermis. There was a slight perivascular infiltration of round cells. The small arteries showed no change except slight intimal thickening, and

the medium-sized arteries showed slight medial degeneration, but both changes were within the normal limits of change due to age. The veins were entirely normal. Simple atrophy of bone was present, with narrowing of the trabeculae and reduction of osteoclasts. The areas between the irregular trabeculae of the third proximal phalanx were replaced by dense collagenous connective tissue at the point of the severed bone, and beginning constriction was present on the proximal two phalanges of the fourth toe. The nerves of the toe had a normal structure.

Examination of the amputated leg revealed a necrotic ulcer 2.5 cm. in diameter over the lateral malleolus and a 2 cm. ulcer over the fifth tarsometatarsal joint. The sites of amputation of the first four toes showed incomplete healing. Dissection of the vessels showed the popliteal artery to be moderately sclerotic without appreciable narrowing. The anterior tibial artery was sclerotic and 60 per cent narrowed. The orifice of the peroneal artery was narrowed. The posterior tibial artery was occluded by a hyaline thrombus 4 cm. distal to the bifurcation. Microscopic sections revealed an atheromatous plaque in the popliteal artery causing a small amount of narrowing. No calcification was present in the wall. Section of the anterior tibial artery showed sclerosis of the wall with medial ossification. Section of the posterior tibial artery showed a hyaline thrombus which was partially canalized. The findings were therefore those of arteriosclerosis with arterial thrombosis in the larger vessels of the leg, but the blood vessels of the third and fourth toes were within normal limits of age change for the patient. Thus vascular changes alone would not account for the developing spontaneous amputations. Hyperkeratosis and parakeratosis were present in the epidermis at the site of the constriction of the toes, and contracting collagenous fibers had completely severed the proximal phalangeal bone of the third toe and were constricting the proximal phalangeal bones of the fourth toe, resulting in the clinical manifestation of ainhum.

ETIOLOGY OF AINHUM

Numerous theories have been advanced as to the cause of ainhum but none is satisfactory. It seems reasonable to consider ainhum as a manifestation of various diseases rather than a separate disease.

Local Diseases of the Skin.—Spinzig [3] noted that in 8 of the reported cases of ainhum in the United States corns or calluses were localized at the point of constriction. Unna has been cited as considering ainhum a local ring form scleroderma causing endarteritis and rarefying osteitis. Despetits and Corre [4] and da Silva Lima [1] also favored this idea, and Grschebin [5] reported Barthelemy, Besmer and Leistrkow as considering ainhum to be on a basis of local scleroderma. In Snider's [6] case, presented in 1929, there was not complete agreement as to the diagnosis of ainhum, and the possibility of the condition having been caused by a fungous infection was mentioned. In reference to this case Tobias commented that "ainhum includes numerous diseases of the small toes." Later that same year Gross [7] presented a case of ainhum which he believed due to trauma and trophoneurosis.

3. Spinzig, E. W.: Am. J. Roentgenol. **42**:246, 1939.

4. Scheube, B.: Die Krankheiten der warmen Länder, Jena, Gustav Fischer, 1900, p. 632.

5. Grschebin, S.: Urol. & Cutan. Rev. **40**:98, 1936.

6. Snider: Arch. Dermat. & Syph. **20**:139, 1929.

7. Gross: Arch. Dermat. & Syph. **21**:874, 1930.

Nonlocalized Diseases of the Skin.—The occurrence of ainhum has been reported several times associated with widespread disease of the skin. Hyde and Montgomery [8] in 1904 referred to 3 white patients with palmar and plantar keratoses in whom ainhum had also developed. They considered ainhum as scleroderma annulare "originating in the causes found effective in the ordinary types of scleroderma." Pavlovskoi and Karishevoi [9] reported a case in which ainhum developed in a 17 year old Russian girl with palmar and plantar keratoses, and Wigley,[10] a case of ainhum-like constriction of the fingers in a 10 year old white girl with palmar and plantar keratoses. Grschebin [5] cited Pardo-Castello and Mestra, who saw 6 patients with ainhum over a fifteen year period and expressed the opinion that ainhum can be caused by various conditions. One of their patients had leprosy and another keratodermia. The latter patient came from a family in which 7 members had keratodermia, and in 3 of these this had resulted in ainhum. Grschebin [5] reported the case of an 18 year old Russian girl with general ichthyosis and keratosis palmaris et plantaris in whom ainhum of both little toes developed. He also cited Pavlowskaja-Karyschewa, who reported a case in which ainhum occurred in a 12 year old Russian girl six years after the development of scleroderma, and Pospelow, who described a patient with sclerodactylia in whom ainhum developed. Stelwagon [12] observed ainhum in a 28 year old man with a cutaneous condition thought to be pityriasis rubra pilaris; there had been loss of one small toe, and there were beginning constrictions of other toes and one little finger. The development of ainhum in these varied dermatologic conditions adds further support to the view that ainhum is a symptom and not a disease.

Injury and Mechanical Irritation.—Heitzmann [13] favored the idea of self-induced trauma as the cause of ainhum since he had observed cases in which local ligatures or strings were important etiologic factors. Eyles [14] favored the theory of an injury of the digitoplantar fold in which there was local introduction of foreign material causing hyperplasia of the epidermis, with subsequent pressure on the vasomotor nerves producing the trophic phenomena. Manson [15] also favored the theory of injury and irritating foreign matter. In addition to the unguarded lateral position of the fifth toe and the greater likelihood of trauma Paterson [1] thought the obliquity of the fourth and fifth flexor tendons of Negroes might be a factor in the traumatic origin of ainhum.

8. Hyde, J. N., and Montgomery, F. H.: Ainhum, in A Practical Treatise on Diseases of the Skin, ed. 4, Philadelphia, Lea Brothers & Co., 1897, p. 598; ed. 7, Philadelphia, Lea & Febiger, 1904, p. 608.

9. Pavlovskoi and Karishevoi: Abstracted, J. Cutan. Dis. **36**:133, 1918.

10. Wigley, J. E. M.: Brit. J. Dermat. **41**:188, 1929.

11. Footnote deleted by the author.

12. Stelwagon, H. W.: A Treatise on Diseases of the Skin, ed. 8, revised, Philadelphia, W. B. Saunders Company, 1918, p. 656.

13. Heitzmann, C.: Tr. Am. Dermat. A. **5**:49, 1881.

14. Eyles, C. H.: Lancet **2**:576, 1886.

15. Manson, P.: Tropical Diseases, ed. 1, revised, London, Cassell & Co., 1903, p. 725.

Infection.—Shepherd [16] expressed the belief that ainhum is on a basis of local infection, and in 1887 he predicted that an "ainhum bacillus" would be found, but this concept of a specific etiologic organism never proved true. In the same year Horowitz [17] reported a case in which he concluded that infection was the important factor. Wellman [18] expressed the belief that infestation of the skin by Sarcopsylla penetrans with associated infection had caused the ainhum in his cases, and Babler [19] and Castellani and Chalmers [20] also favored a parasitic origin of the condition.

General Diseases.—Leprosy and syphilis have both been reported as having caused ainhum. Grschebin [5] in 1922 noted ainhum in 2 patients in the Astrakhan leprosarium. Zambaco-Pacha and Pardo-Castello and Mestre are cited as having also made the same clinical observation. Other workers have noted that leprosy more frequently produces a different clinical picture since anesthesia of the foot permits severe injuries to occur, with ulcers that extend into the joints of several of the toes.

Bharucha [21] observed ainhum in a Hindu with syphilis and Wright [22] in a man with syphilis. Both investigators expressed the belief that syphilis caused the ainhum.

Metabolic Disturbances.—Numerous investigators have seen ainhum which they believed to be on either a neurotrophic or a vascular basis. Shepherd [16] expressed the opinion that a trophic disturbance of the nerve centers had caused ainhum in a patient he had seen. Later other investigators, including Sheube,[4] Matas,[23] Abbe,[24] Pusey [25] and Stelwagon,[12] considered a neurotrophic disorder to be the cause of ainhum. Weinstein [26] reported that he had found the sensation of the affected toes diminished or increased but never entirely lost. Sutton [27] described a child with ainhum who died of an obscure nervous disorder; at autopsy degenerated areas in the cord and peripheral neuritis were observed. Welch [28] found the tactile sense slightly impaired in the distal part of the toe, although the reflexes were normal. He expressed the belief that ainhum is due to trophoneurosis plus a traumatic element.

16. Shepherd, F. J.: Am. J. M. Sc. **93**:137, 1887.

17. Horowitz, O.: Med. & Surg. Reporter **56**:649, 1887.

18. Wellman, F. C.: J. Trop. Med. **11**:117, 1908.

19. Babler, E. A.: Ann. Surg. **48**:110, 1908.

20. Castellani, A., and Chalmers, A. J.: Manual of Tropical Medicine, New York, William Wood & Company, 1910, p. 1148.

21. Bharucha, E. S.: Indian M. Gaz. **52**:403, 1917.

22. Wright, L. T.: Urol. & Cutan. Rev. **28**:135, 1924.

23. Matas, R.: Tr. Am. S. A. **14**:483, 1896

24. Abbe, T.: M. Rec. **79**:478, 1911.

25. Pusey, W. A.: Principles and Practice of Dermatology, ed. 3, D. Appleton and Company, 1917.

26. Weinstein, H.: Proc. Canal Zone M. A. **4**:110, 1911.

27. Sutton, R. L.: Diseases of the Skin, ed. 9, revised, St. Louis, C. V. Mosby Company, 1935, p. 624.

28. Welch, R. S. G.: U. S. Nav. M. Bull. **21**:352, 1924.

Guimares [29] expressed the opinion that the cause of ainhum is a contracture of arteries that nourish the toes. This subsequently results in a circulatory deficiency and spontaneous amputation of the toes. According to Ashley-Emile,[30] strain on the obliquely placed fourth and fifth flexor tendons produces atrophy of the nerve followed by degeneration of the muscle and rotation of the toe, which produces strangulation of the blood vessels. In the present reported case, there was a vascular impairment of the extremity, but a study of the arteries of the blood vessels near the area of ainhum is convincing that the initial corns and irritation were probably important etiologically. The absence of this phenomenon in the presence of the high incidence of gangrene of the extremities is further evidence of this. No case similar to the present reported case could be found among published instances of gangrene of the extremities.

Doubt regarding the occurrence of ainhum as an independent disease has been expressed by the previously cited authors, who have believed that in their reported cases the lesion was a manifestation of one or another of various diseases. Thus ainhum may be due to a local ring form scleroderma or associated with the pathologic conditions that arise with corns, calluses, fungous infections, palmar and plantar keratoses, keratodermia, ichthyosis, scleroderma, pityriasis rubra pilaris, leprosy, syphilis or neurotrophic or vascular disorders. When no specific disease is demonstrable, the ainhum probably results from scarring and contracture in the digitoplantar fold as a result of trauma and infection. The fibrogenetic character of the Negro further augments this condition and explains the greater incidence in members of that race. Grschebin expressed this view well when he stated, "We support this standpoint, that ainhum is a symptom and not an independent disease and that it may be observed in a number of diseases with similar pathogenesis." More recently Ash and Spitz [31] have stated that "ainhum is probably not a specific entity but a complication that may arise." The present case supports this view and adds additional evidence that ainhum is a symptom rather than an independent disease.

SUMMARY

The clinical picture of ainhum with a toe being spontaneously amputated by a constricting fibrous ring at its base may occur as a symptomatic manifestation of many local or general diseases. A case is presented in which ainhum occurred in a white patient with diabetes mellitus, affording further evidence that ainhum is a symptom rather than a distinct disease.

29. Dell'Orto, J.: New Orleans M. & S. J. **8**:516, 1880-1881.

30. Ashley-Emile, L. E.: J. Trop. Med. **8**:33, 1905.

31. Ash, J. E., and Spitz, S.: Ainhum, in Pathology of Tropical Diseases, Philadelphia, W. B. Saunders Company, 1945, p. 344.

General Reviews

METASTATIC CALCIFICATION

R. M. MULLIGAN, M.D.
DENVER

THE PHENOMENON of metastatic calcification has been known for nearly a century.[1] A comprehensive modern review of the subject has not been found. To the earlier literature Askanazy[2] contributed an analysis of the human cases reported up to 1901, to which he added 2 cases of his own. He particularly emphasized bone disease, neoplastic and otherwise, as an underlying cause of metastatic calcification and pointed out the association of renal disease in some of these cases. Since that time chronic renal disease, primary neoplasms of the parathyroid glands and hypervitaminosis D have been demonstrated to cause metastatic calcification in man. Some human cases with no definitely proved etiologic basis have been recorded. Experimentally, vitamin D, parathyroid extract and mineral diets have been employed to produce metastatic calcification in animals. The main purposes of this review are to analyze the reported human cases in which anatomic findings were recorded, to correlate significant clinical details when possible, to summarize available experimental evidence, especially with regard to studies of tissues, and to elaborate the mechanisms involved in metastatic calcification.

Before attempting such a review, one should formulate some definition of the term "metastatic calcification." Virchow[1] thought that the calcium deposits seen in the lungs and the stomach in his cases represented direct calcification of the tissue by which lime salts of the blood penetrated and filled up the tissue. Wells[3] stated that in this condition calcium salts are deposited throughout the body in apparently perfectly normal tissues, but especially in the lungs, the kidneys and the gastric mucosa, sites where excretion of acid causes a more alkaline reaction in the tissues concerned and results in the precipitation of calcium salts, less soluble because of the lowered concentration of hydrogen ions in these tissues. In other words, metastatic calcification is a condition in which calcium

From the Department of Pathology, University of Colorado School of Medicine.

1. Virchow, R.: Virchows Arch. f. path. Anat. **8**:103, 1855.
2. Askanazy, M., in Festschrift für Max Jaffe, Braunschweig, F. Vieweg & Sohn, 1901, pp. 208-240.
3. Wells, H. G.: Arch. Int. Med. **11**:721, 1911.

salts are deposited in tissues previously normal but with a tendency toward alkalinity.

METASTATIC CALCIFICATION AND BONE DISEASE

The original case of metastatic calcification was that described by Virchow.[1] The patient was a young girl who suffered from rheumatism, loss of weight and bone pain. Cancerous nodules were found in the skeletal bones, chiefly in the vertebrae and the skull; calcium deposits were present in the lungs and the stomach, and renal calculi were found. His second case [1] will be described in the next section of this review. His third case [1] was that of a 15 year old girl who had two recurrences of a myxosarcoma of the left cheek during three months. Metastatic nodules were observed in the skull bones, the brain, the lungs and the intestine and calcium deposits in the lung. His fourth patient [1] was a youth 19 years old, who had been afflicted for sixteen years with necrosis of the proximal end of the left femur. Operation for removal of a sequestrum was followed by widespread erysipelas. Calcium was deposited in the stomach, and amyloidosis involved the spleen. His fifth case [1] concerned a man 73 years old, who was operated on for carcinoma of the lip and the cervical lymph nodes. The lungs, the kidneys, the other organs, the clavicles and a rib were sites of metastases. The lungs contained calcium deposits. Also present in Virchow's third, fourth and fifth cases was active degenerative nephritis in the late second or beginning third stage.

The first case reported by Virchow in a second paper [4] was that of a woman 50 years old, who had a mammary cystosarcoma resected and recurring during two years. The thoracic wall and the adjacent lung were involved by neoplastic tissue. Metastases were seen in the following sites: lungs, mediastinum, liver, ribs, vertebrae, pelvic bones, dura, skull, cavernous sinus. Calcium deposits marked the lungs, the stomach, the kidneys, the rectum, the dura and the internal carotid artery.

Küttner's [5] patient was a 19 year old woman who had abdominal pain and diarrhea for two months. Her spleen and liver were enlarged. She had fever, increasingly rigid peripheral arteries and terminal meningitis. Autopsy revealed: extensive rarefying osteitis or "scrofulous caries" of the thoracolumbar vertebrae; caseous tuberculosis of the meninges, the spleen, the liver and the kidneys; calcification of the systemic arteries; amyloidosis of the spleen and the liver.

The 48 year old man described by Litten [6] suffered from loss of weight, anorexia, and pain and swelling of the extremities for a few months. He exhibited anasarca, a right submaxillary tumor, multiple

4. Virchow, R.: Virchows Arch. f. path. Anat. **9**:618, 1856.
5. Küttner: Virchows Arch. f. path. Anat. **55**:521, 1872.
6. Litten, M.: Virchows Arch. f. path. Anat. **83**:508, 1881.

pigmented nevi on the face, the neck and the trunk, dyspnea, oliguria and a low blood pressure. Metastatic alveolar round cell sarcoma, probably arising in a cancerous cutaneous melanoma, involved all viscera, especially the stomach, the intestines, the peritoneum, the heart, the kidneys, the vertebrae and the skeletal muscles. The kidneys and the cutaneous arteries contained calcium deposits.

Roth [7] described a man 29 years old in whom osteomyelitis of the phalanges developed after traumatic amputation of the fingers. Incision and drainage were followed by nausea, vomiting, fever, delirium, drowsiness and albuminuria. The heart and the stomach were calcified, and the left renal vein and branches were thrombosed.

In a woman 26 years old, Haskoveč [8] found cancer of the vagina, osteoporosis of bones caused by "capillary emboli" of cancer cells and calcification of the lungs and the kidneys.

A 15 year old youth described by Czech [8] had a partly calcified sarcoma of the prostate gland, a paravesical abscess eroding a pubic bone, calcific deposits in the lungs and parenchymatous degeneration of the kidneys.

Plaue [8] examined a woman 49 years old who had neoplasms of unspecified nature in the skull, the twelfth thoracic vertebra and a rib, calcification of the left lung and chronic interstitial nephritis.

Heller [8] saw a patient with cancer of a vertebra, calcium deposits in the lungs and diseased kidneys. The renal lesion was not defined.

Kockel's [9] first case was that of a woman 35 years old who had had a vaginal hysterectomy for cervical cancer three years before. For one year she was afflicted with gradual paralysis of the lower extremities. Marasmus, anemia and sacral decubitus were terminal. Autopsy showed: metastatic squamous cell carcinoma of the thoracolumbar vertebrae, the pelvic bones, the liver, the lungs and the pleura; calcification of the lungs; calculi of the left kidney; bilateral hydronephrosis; organizing and recent pneumonia; cancer cell embolism and thrombosis of pulmonary arteries. His second case [9] concerned a woman 54 years old, who had undergone hysterectomy for cervical carcinoma. Autopsy disclosed metastatic squamous cell carcinoma in the left iliac bone, in the lumbar vertebrae and in the lungs, calcium deposits in the lungs and emboli of cancer cells in pulmonary arteries.

The first case of Davidsohn [10] was that of a man 27 years old, who had a sarcoma of the first sacral vertebra with invasion of the adjacent vertebrae and of the pelvic bones and metastases in the lungs. Calcium deposits marked the heart, the lungs and the kidneys. His second

7. Roth, M.: Cor.-Bl. f. schweiz. Aerzte **14**:226, 1884.
8. Cited by Askanazy.[2]
9. Kockel, R.: Deutsches Arch. f. klin. Med. **64**:332, 1899.
10. Davidsohn, C.: Virchows Arch. f. path. Anat. **160**:538, 1900.

patient [10] was a 32 year old man with a gastric carcinoma metastasizing to the pelvic bones, a humerus and a femur, calcification of the left lung, the stomach and the kidneys, and fat embolism of the lungs.

Askanazy's first patient [2] was a man 37 years old, with a large round cell sarcoma (obviously a cancerous melanoma) of the right thigh, which metastasized to the following sites: right inguinal lymph nodes, skin, heart, liver, left adrenal gland and kidney, pancreas, sternum, ribs, dura, frontal bone, second lumbar vertebra, left iliac bone. Also observed were calcification of the stomach, pulmonary emphysema, purulent bronchitis, hemorrhagic nephritis and thrombosis of renal veins. His second case [2] concerned a woman 54 years old, who had a goiter for five years. For one year, after trauma of her back, she suffered sacral pain. For three weeks she had renal colic and passed two calcium sulfate stones. Terminally her urine contained leukocytes and coliform organisms, and she was afflicted with vomiting, fever, and renal pain in the left side. In the ribs, the vertebrae and the sternum was found "progressive bone atrophy," characterized by osteoclasis, osteoporosis and fibrosis of marrow. Also present were carcinoma of a substernal thyroid gland with metastases in the lungs, calcium deposits in the lungs and the kidneys, calcified thrombi in the pulmonary arteries, renal calculi, purulent pyelitis and organizing pneumonia.

Bender's first patient [11] was a youth 14 years old, who had arthritis of the shoulders, the elbows, the hands, the ankles and the feet. A nodule removed from the patella for biopsy was diagnosed as lymphosarcoma. Also observed were: a primary round cell sarcoma of the periosteum of the left femur with metastases in ribs, vertebrae, femurs, tibias, fibulas, humeri, lymph nodes, kidneys and right testis; calcification of the lungs, the stomach, the liver and the kidneys; lobular pneumonia. His second patient [11] was a man 51 years old, who suffered from sacral pain, melena, constipation and paresthesias of the legs for three months. This patient was emaciated, dipsomaniac and unable to walk. He showed deformities of the thoracolumbar vertebrae, paralysis of the lower extremities, sacral decubital ulcer and albuminuria. Plasma cell myeloma involved skull, ribs, vertebrae, left clavicle, pelvic bones, sacrum, scapula, right humerus and right femur. Calcification of the lungs, compression of the sacral plexus, embolism of a branch of the right pulmonary artery and bilateral cystoureteropyelonephritis were also present.

Stokvis [12] described a 39 year old man with Bence Jones protein in the urine and the feces. A diffuse bone disease, called "osteosarcomatosis," but probably multiple myeloma, and calcification of the kidneys were found.

---

11. Bender, O.: Deutsche Ztschr. f. Chir. **63**:370, 1902.
12. Stokvis, B. I., cited by Parkes-Weber, F. A.: Med.-Chir. Tr. **86**:395, 1903.

For seven months a man 50 years old, described by Scheele and Herxheimer,[13] suffered from pain in the chest, the back, the joints and the muscles. Two months before, he had bronchitis and pneumonia. He showed fever, recurrent pneumonia, spontaneous fracture of the left femur, albuminuria and swelling of the right cervical and left inguinal regions. Multiple myeloma of the left femur, the ribs, the sternum, the clavicles and the vertebrae, calcification of the kidneys, bronchopneumonia and nephritis were found.

A woman 19 years old, described by Lazarus and Davidsohn,[14] suffered from fever, night sweats, pain in the feet, the right leg and the shoulders, and swelling of the right knee. She exhibited a systolic murmur, paralysis of the left abducens nerve, dyspnea, prostration, splenomegaly, albuminuria and pulmonary rales. A meningeal sarcoma invaded the skull bones, and the heart, the lungs, the stomach, the kidneys and the aorta were calcified.

Huebschmann[15] reported the case of a woman 26 years old, who showed the following conditions: a carcinoma of the vagina with erosion of the pelvic bones, invasion of the bladder and the rectum, and compression of the ureters; calcification of the lungs and the kidneys; calcific thrombosis of an artery and a calcified infarct in the lower lobe of the right lung, and bronchopneumonia.

For five years a woman 36 years old, described by Tschistowitsch and Kolessnikoff,[16] suffered from pains in the neck, the trunk and the extremities. For six months she had dyspnea, vomiting and constipation. She showed elevated vital signs, emaciation, albuminuria with Bence Jones protein, and terminal pneumonia. Autopsy disclosed: myeloblastic myeloma in the ribs, the sternum, the clavicles, the ilium and the vertebrae; calcification of the heart, the systemic arteries, the lungs, the stomach and the kidneys; organizing pneumonia.

For three months, following trauma, a boy 12 years old, described by Jadassohn,[17] had pain in the trunk, polydipsia, loss of weight and vomiting. The following abnormalities were observed clinically: cutaneous nodules over the elbows and knees; subcutaneous stripes; vesicles, pustules and abscesses in the skin; high grade destruction of the pelvic bones, revealed on roentgen examination; a blood culture showing staphylococci; a terminal high fever. Autopsy disclosed: osteomyelitis of the right ilium and of the ribs; rarefaction of the left ilium and of the tibial epiphyses; calcium deposits in the heart, the lungs,

13. Scheele and Herxheimer: Ztschr. f. klin. Med. **54**:57, 1904.

14. Lazarus, P., and Davidsohn, C.: Ztschr. f. klin. Med. **60**:314, 1906.

15. Huebschmann, P.: Centralbl. f. allg. Path. u. path. Anat. **19**:737, 1908.

16. Tschistowitsch, T., and Kolessnikoff, H.: Virchows Arch. f. path. Anat. **197**:112, 1909.

17. Jadassohn, J.: Arch. f. Dermat. u. Syph. **100**:317, 1910.

the splenic arteries, the kidneys and the skin; staphylococcic aortic valvulitis; multiple pulmonary, myocardial and renal abscesses (left kidney).

Pari [18] reported the case of a woman 25 years old, with the following conditions: carcinoma of the uterine cervix; metastases in lumbar vertebrae, the ovaries, the liver and lumbar lymph nodes; extension to the ureters with obstructive hydronephrosis; extension to the bladder with perforation; calcification of the lungs; organizing pneumonia.

A man 25 years old, described by Versé,[19] had chronic myelogenous leukemia for thirty months. Typical lesions involved the marrow, the spleen and the liver. Also present were: extensive destruction of bone; calcification of the heart, the systemic arteries, the lungs and the kidneys; mural thrombosis of the left auricle.

Schober [20] examined a patient with a destructive inflammatory process, probably osteomyelitis, of the calvarium and calcification of the heart and the lungs.

Wells [21] reported the case of a 30 year old man who had: myelogenous leukemia with typical lesions in the sternum, the clavicles, other bones, the liver, the kidneys and other organs; calcification of the heart, the coronary arteries, the lungs and the kidneys; a patent foramen ovale.

For two months the 52 year old woman described by Froboese [22] had a tender mass in the right lumbar region. A severely pyonephrotic right kidney was removed at operation. She began to have swollen, painful feet, pallor, enlargement of the heart, bronchitis and albuminuria. A multiple myeloma (*Erythroblastom*) was found involving the sternum, the ribs, the vertebrae, the femurs and other bones, with halisteresis, osteoporosis and osteoclastosis, and calcification of the lungs and the kidneys.

Schulze's [23] patient was a boy 11 years old, who suffered pain and limitation of motion of the vertebrae and of the extremities for one year. He displayed emaciation, pallor, weakness, thoracic scoliosis, a rigid spine, rachitic teeth, cutaneous and tendinous calcium deposits, hard peripheral arteries, calcified arteries in the extremities by roentgenogram, slight albuminuria and terminal cardiac failure. Osteopetrosis (Albers-Schönberg disease) of the skeletal bones was found, and calcium deposits in the heart, the systemic arteries, the lungs, the stomach, the kidneys and the periarticular structures of the hip, the knee and the vertebral joints.

18. Pari, G. A.: Virchows Arch. f. path. Anat. **200**:199, 1910.
19. Versé, M.: Verhandl. d. deutsch. path. Gesellsch. **14**:281, 1910; Centralbl. f. allg. Path. u. path. Anat. **21**:459, 1910.
20. Schober, cited by Stumpf: Centralbl. f. allg. Path. u. path. Anat. **25**:801, 1914.
21. Wells, H. G.: Arch. Int. Med. **15**:574, 1915.
22. Froboese, C.: Virchows Arch. f. path. Anat. **222**:291, 1916.
23. Schulze, F.: Mitt. a. d. Grenzgeb. d. Med. u. Chir. **36**:243, 1923.

For two years the 46 year old woman observed by Barr and Bulger [24] had night sweats and lassitude. Examination showed: fever; emaciation; pallor; rarefaction of the ribs, the lumbar vertebrae and the pelvic bones by roentgenogram; a hemoglobin content of 48 per cent; erythrocytes 3,250,000 per cubic millimeter; serum calcium 16 mg. and serum phosphorus 3.7 mg. per hundred cubic centimeters; blood nonprotein nitrogen 70 mg. per hundred cubic centimeters; albuminuria with hyaline and granular casts; excretion of 51 per cent of injected phenolsulfonphthhalein in two hours; serum calcium 17.8 mg. per hundred cubic centimeters with a high calcium intake; terminal pneumonia. Autopsy revealed: plasma cell myeloma in the ribs, the clavicles and the vertebrae; calcification of the lungs, the stomach and the kidneys; bronchopneumonia; chronic nephritis; calculi of the gallbladder and the cystic duct.

The 19 year old woman described by deSanto [25] had suffered weakness, anorexia and loss of weight for one year. Ten months before she was admitted to the hospital she had an acute rash, pruritus, splenomegaly and a blood picture typical of myelogenous leukemia. Examination showed: pallor; emaciation; albuminuria; a basal metabolic rate of + 75 per cent; 21,200 to 1,200,000 blood leukocytes per cubic millimeter, with varying percentages of immature neutrophilic granulocytes; terminal pulmonary rales, toxemia and coma. Typical lesions of myelogenous leukemia involved the bone marrow, the spleen, the liver, the kidneys and lymph nodes. Calcification was present in the heart, the systemic arteries, the lungs and the kidneys. The lungs contained multiple arterial emboli and infarcts.

Egoville [26] reported the case of a woman 30 years old. Her fourth pregnancy had begun nine months before. For three months she noticed progressive weakness and severe pain in the shoulders, the back and the lumbar region. One month before examination she was delivered of a macerated fetus, and roentgenograms showed punched-out areas in the calvarium, the vertebrae, the femurs and the humeri. Biopsy of a rib showed metastatic adenocarcinoma. The serum calcium was 18.2 mg. and the serum phosphorus 9 mg. per hundred cubic centimeters, and the total serum protein was 5.5 Gm. per hundred cubic centimeters. Gastric analysis revealed achlorhydria. Adenocarcinoma of the right breast with metastases to vertebrae, ribs, liver and lymph nodes and calcification of the heart, the lungs and the kidneys were demonstrated at autopsy.

For two months the 13 year old girl described by Grayzel and Lederer [27] had pain in the thighs, radiating to the ankles and the feet,

24. Barr, D. P., and Bulger, H. A.: Am. J. M. Sc. **179**:449, 1930.
25. DeSanto, D. A.: Am. J. Path. **9**:105, 1933.
26. Egoville, J. W.: Arch. Path. **26**:1047, 1938.

(Footnotes continued on next page)

pain in the flanks, vomiting and loss of weight. Examination disclosed: a palpable right kidney; a tender left twelfth rib; rarefaction and mottling of the shafts of the fibulas and of the thoracolumbar vertebrae and punched-out areas in the humeri; slight albuminuria; a phenolsulfonphthalein test with excretion of 27 per cent; a blood hemoglobin content of 60 per cent; 3,400,000 erythrocytes per cubic millimeter; 6,300 leukocytes per cubic millimeter, with 85 per cent neutrophils and 15 per cent lymphocytes; plasma chlorides 424 mg., blood nonprotein nitrogen 101 mg., serum albumin 3.5 Gm., serum globulin 2.8 Gm., serum cholesterol 197 mg., serum calcium 20.6 mg., serum phosphorus 4.7 mg., serum phosphatase 4.5 units [27a] and blood sugar 134 mg. per hundred cubic centimeters; carbon dioxide–combining power 53.5 volumes per cent. Also found were aleukemic myelosis, involving ribs, vertebrae, spleen, liver, lymph nodes, kidneys and thymus, and calcification of the heart and the lungs.

Wells and Holley [28] reported the case of a man 59 years old, who noticed an enlarging head for nine years, bowed legs for five years, weakness and loss of weight for four years, pain and stiffness in the left hip and knee for three years and alopecia, diplopia and decreased visual acuity for eight months. Examination showed: a large sternum; flared ribs; a barrel chest; a systolic murmur; pulmonary rales; spinal kyphosis; enlarged, thickened, moth-eaten calvarium, mandible, and bones of extremities, variation in the density of the vertebrae and enlarged pelvic bones by roentgenogram; serum calcium 10 to 11.8 mg., serum phosphorus 3.7 to 4.5 mg., serum potassium 16.7 to 17.9 mg. per hundred cubic centimeters; a blood p_H of 7.3 to 7.45. He received 5,000,000 units of viosterol in two weeks and died of terminal bronchopneumonia. Osteitis deformans was found in the skull, the sternum, the ribs, the vertebrae, the iliac bones, the left femur and the left tibia, and calcium deposits in the heart, the lungs, the stomach, the kidneys and the skin.

Summary.—Of 35 patients having some type of bone disease and metastatic calcification, 17 were of the male and 18 of the female sex. The ages of 34 were between 11 and 73 years, the disease occurring before the age of 40 in 23 and after the age of 60 in only 1. The lesions of the bones represented metastatic carcinoma in 7 cases, multiple myeloma in 6, osteomyelitis in 5, myelogenous leukemia and unspecified cancer in 4 each, metastatic sarcoma in 3, primary sarcoma and metastatic melanoma in 2 each, and tuberculosis, "progressive bone atrophy," osteopetrosis (Albers-Schönberg disease) and osteitis deformans in 1 each. In 1 case of osteomyelitis [8] metastatic bone sarcoma was probably

27. Grayzel, D. M., and Lederer, M.: Arch. Int. Med. **64**:136, 1939.

27a. In all instances in which the type of unit is not stated this is due to the fact that the type was not specified in the article reviewed.

28. Wells, H. G., and Holley, S. W.: Arch. Path. **34**:435, 1942.

present. In Bender's [11] first patient with primary sarcoma of bone, probably Ewing's sarcoma, metastatic bone sarcoma was widespread. As a contributing cause of metastatic calcification, some type of nephropathy was found in 15 cases, including 3 reported by Virchow,[1] those of Küttner,[5] Czech,[8] Plaue [8] and Heller,[6] Kockel's [9] first case, those of Askanazy,[2] Bender's [11] second case and those of Scheele and Herxheimer,[13] Jadassohn,[17] Pari [18] and Barr and Bulger.[24] In the other 22 cases nephropathy other than renal calcification was not a factor so far as could be determined from the available protocols. Associated dystrophic calcification of the lungs was definitely a factor to be considered in both of Kockel's [9] cases, Askanazy's [2] second case, Huebschmann's [15] case and Pari's [18] case. The parathyroid glands were examined in only 4 cases [29] and were found to be normal. Hypervitaminosis D probably played some role in the calcification of the tissues of the patient observed by Wells and Holley.[28]

Calcium deposits were found in the lungs in 30 cases, most often in alveolar walls, veins, arteries, capillaries, bronchi and bronchioles. Elastic fibrils in alveolar walls, veins and arteries were often the sites of calcification. The veins were usually more heavily calcified than the arteries. Less common locations for calcific deposits were the stroma, the bronchial cartilages and the lumens of alveoli and blood vessels.

In 21 cases calcium deposits were found in the kidneys, most often in the lumens, cells and basement membranes of convoluted and collecting tubules and, to a much less extent, of Henle's loops. Also calcified with significant frequency were the renal stroma and the basement membranes of glomeruli. Other less common locations for calcium deposits included arteries, arterioles and capillaries. Thrombosis of the renal veins was observed in 2 instances,[30] but calcium was not described in the renal veins in any case.

In 13 cases the heart contained calcium deposits, preponderantly in the endocardium of the left auricle, involving especially elastic fibrils. In a few cases the endocardium and the myocardium of the left ventricle showed calcified elastic fibrils and muscle fibers. The mitral and aortic valves were also involved by calcification in a few instances. The right chambers of the heart were little if any involved.

In 12 cases the systemic arteries were calcified, including the abdominal aorta, the coronary, mesenteric, femoral, hepatic, renal, splenic, carotid, pancreatic and cutaneous arteries, and the arteries of the extremities. The calcium salts were found as fine granules or plaques in an otherwise normal intima, often in relation to the internal elastic lamella, and in the elastic fibrils, or sometimes in the muscle fibers, of the media.

29. Barr and Bulger.[24] Egoville.[26] Grayzel and Lederer.[27] Wells and Holley.[28]
30. Askanazy.[2] Roth.[7]

In 10 cases the stomach contained calcium deposits, most notably in the interglandular stroma of the fundic mucosa and to a lesser degree in gland cells and lumens, and in capillaries.

Miscellaneous sites of calcification included the skin,[31] the rectum,[4] the dura,[4] periarticular structures [28] and the liver.[11] In Bender's first case [11] the calcification of the liver was most likely dystrophic in nature.

Chemical analysis showed calcium carbonate in the lungs of 5 patients,[32] in the hearts of 3,[33] in the kidneys of 2 [34] and in the gastric mucosa,[7] the skin [17] and the renal calculi [11] of 1 each. Calcium phosphate was found in the lungs,[35] the gastric mucosa,[10] the heart,[21] the kidney [21] and in renal calculi.[1] Calcium sulfate was found in the renal calculi of 1 patient.[2]

METASTATIC CALCIFICATION AND CHRONIC RENAL DISEASE

The second case of metastatic calcification reported by Virchow [1] was that of a woman 43 years old who had had pleuritis two months before and then suffered from bleeding hemorrhoids. The clinical findings were: fever; anasarca; bronchitis; abdominal pain; polyuria; casts, erythrocytes and leukocytes in the urine; terminal erysipelas, vomiting and dyspnea. Autopsy revealed active degenerative nephritis in the late second or beginning third stage of the disease; calcification of the left lung; pulmonary edema; an infarct of the left kidney; left serofibrinous pleuritis.

The kidneys of a man 44 years old, described by Stade,[8] showed interstitial nephritis, and both lungs and kidneys contained calcium deposits.

Bryant and White [36] recorded the case of a 6 month old boy who had suffered from loss of weight, weakness and constipation for three months. He showed a scruffy, alopecic scalp, a few basal rhonchi in the lungs, vomiting, diarrhea, and terminal gangrene of the right foot. Autopsy disclosed: obstructive cystoureteropyelonephritis with hydronephrosis; extreme phimosis; calcification of the heart and the systemic arteries; nodular tuberculosis of the lungs, the bronchial lymph nodes and the spleen; thrombosis of the right anterior tibial artery.

In a woman 36 years old, Schmidt [37] found: chronic interstitial nephritis; renal endarteritis; calcification of the heart, the systemic arteries, the splenic veins, the lungs and the stomach; mural thrombosis of the cardiac ventricles; embolic hemorrhagic infarcts of the upper lobes

31. Jadassohn.[17] Wells and Holley.[28]
32. Kockel.[9] Bender.[11] Jadassohn.[17] Pari.[18] Wells.[21]
33. Roth.[7] Jadassohn.[17] Wells.[21]
34. Roth.[7] Wells.[21]
35. Bender.[11] Tschistowitsch and Kolessnikoff.[16] Wells.[21]
36. Bryant, J. H., and White, W. H.: Guy's Hosp. Rep. **55**:17, 1901.
37. Schmidt, M. B.: Deutsche med. Wchnschr. **39**:59. 1913.

and atelectasis of the lower lobes of the lungs; chronic passive hyperemia of the liver; cholesterolosis of the gallbladder; vaginal ulcers; hyperplastic nodules in the thyroid gland; a calcified right bronchial lymph node.

A man 20 years old, described by Hubbard and Wentworth,[38] had masses around the larger joints of the extremities for eleven months. Examination showed: large calcium deposits around these joints and calcified arteries by roentgenogram; calcified media in a biopsy specimen of a peripheral artery; a serum calcium content of 13.4 mg. per hundred cubic centimeters, falling to 11.9 mg. during three days of a low calcium diet and rising to 12.7 mg. during three days of a high calcium diet; other blood findings typical of progressive chronic nephritis. Autopsy disclosed: severe chronic interstitial nephritis; right hydronephrosis; calcification of the heart, the small peripheral arteries, the periarticular structures, the jejunum and the gastroepiploic omentum; osteitis fibrosa, especially in the skull, the ribs and the vertebrae; hyperplasia of two enlarged parathyroid glands, one inclosing a small adenoma.

Butler's[39] first patient was a Negro woman 38 years old, who suffered from dyspnea and postprandial emesis. Examination showed: emaciation; an enlarged heart with diastolic gallop and apical systolic murmur; rales in the left lung; hepatomegaly; edema of the ankles; a blood pressure of 220 systolic and 160 diastolic; blood urea 5.6 Gm. per liter; albuminuria with many leukocytes; terminal uremia. At autopsy chronic nephritis, calcification of the left lung, edema of the right lung, organizing pneumonia, cardiac hypertrophy and an infarct of one kidney were found. His second patient was a woman 31 years old, who had suffered from headaches, palpitation, bilious attacks and edema of the legs and feet for eight years. Examination showed: poor nurture; an enlarged heart; pulmonary rales; albuminuria; a phenolsulfonphthalein test with excretion of less than 10 per cent; blood urea 1.43 to 3.63 Gm., per liter. Also present were chronic nephritis, calcium deposits in the lungs, edema of the lungs and right fibrinous pleuritis.

Müller[40] recorded the case of a man 20 years old, who had had his right femur amputated for tuberculous osteomyelitis seven years before. For one year he suffered from purulent streptococcic pyelocystitis, and died in uremia. Autopsy disclosed: pyonephrosis of the left kidney; chronic left ureteritis; chronic cystitis; suppurative pericystitis; amyloidosis of the right kidney and of the spleen, the liver and the heart; calcium deposits in the heart, the systemic arteries and the right kidney; tuberculosis of the left lung, the bronchial lymph nodes, the spleen and the liver.

38. Hubbard, R. S., and Wentworth, J. A.: Proc. Soc. Exper. Biol. & Med. **18**:307, 1921.

39. Butler, M.: Proc. New York Path. Soc. **24**:79, 1924.

40. Müller, H.: Klin. Wchnschr. **5**:1703, 1926.

The 27 month old girl described by Lightwood [41] suffered from stunting of growth and intermittent vomiting. She had facial paralysis twenty-two months before; a cardiac murmur one year before, and failure to gain weight, dysphagia, flatulence and inability to walk for one year. For ten days before admission she received 2 minims (0.12 cc.) of a preparation of vitamin D daily. She exhibited dwarfism, retarded mentality, pallor, poor nurture, genu valgum and carious teeth. Examination showed: a systolic blood pressure of 180; hard tortuous arteries in the extremities, visible by roentgen ray; narrowed retinal arterioles; paresis of the right side of the face and of the left external rectus muscle; albuminuria; an average urea concentration of 1.5 per cent in two hours; blood urea 169 mg., blood cholesterol 196 mg., serum calcium 11 mg. and serum phosphorus 6.7 mg. per hundred cubic centimeters; terminal fever. Autopsy revealed: chronic nephritis; calcium deposits in the heart, the systemic arteries, the lungs, the kidneys, the trachea, the parietal pleura, the dura and the tentorium; rickets in the long bones; pulmonary edema; bronchopneumonia.

Albright, Baird, Cope and Bloomberg [42] described a man 23 years old, who suffered from nausea, weakness, vomiting, polyuria and nocturia. He had a blood pressure of 140 systolic and 100 diastolic, 2,500,000 erythrocytes per cubic millimeter, 185 mg. of urea nitrogen per hundred cubic centimeters of blood and albuminuria. Chronic nephritis, calcification of the lungs and the kidneys, osteoporosis and osteoclastosis of the vertebrae and lobular pneumonia were observed.

Platt and Owen [43] reported the case of an 18 year old youth who had been afflicted with weakness, debility, polydipsia, polyuria, nocturia, drowsiness and mental dulness since early childhood. For three years he had genu valgum and anorexia. He displayed dwarfism, emaciation, pallor and pigmented skin raised in hard plaques in the axillary and inguinal regions. Examination showed: an enlarged heart; a blood pressure of 155 systolic and 100 diastolic; albuminuria; a hemoglobin content of 30 per cent; 1,560,000 erythrocytes and 17,900 leukocytes per cubic millimeter; an increased sedimentation rate of the red blood cells; blood nonprotein nitrogen 333 mg., blood urea nitrogen 205 mg., plasma cholesterol 179 mg., serum calcium 6.7 mg. and serum phosphorus 15.1 mg. per hundred cubic centimeters; bone erosion in the spine and sacrum, rickets in the bones of the extremities, and calcification of the arteries and of the pigmented cutaneous areas by roentgenogram. Terminally there were drowsiness, dyspnea, convulsions and uremia, with the blood nonprotein nitrogen 400 mg. per hundred cubic centi-

41. Lightwood, R.: Arch. Dis. Childhood **7**:193, 1932.

42. Albright, F.; Baird, P. C.; Cope, O., and Bloomberg, E.: Am. J. M. Sc. **187**:49, 1934.

43. Platt, R., and Owen, T. K.: Lancet **2**:135, 1934.

meters. Chronic nephritis, calcification of the lungs, the splenic blood vessels, and the skin, cardiac hypertrophy and pulmonary edema were also found.

A boy 14 years old, described by Smyth and Goldman,[44] sustained a streptococcic infection of a finger, followed by lymphangitis, lymphadenitis, bacteremia, nephritis and anemia. Eighteen months later he had a waddling gait and stiffness and weakness of the lower extremities. Two years after the onset of the infection he exhibited a poor posture, emaciation, pallor, increased anteroposterior diameter of the chest and flaring of the costal margins. Examination revealed: an enlarged heart; a systolic murmur; hard radial arteries; a blood pressure of 110 systolic and 56 diastolic; a palpable liver; albuminuria; low urinary output; 3 to 7 per cent excretion of injected phenolsulfonphthalein in two hours; urea clearance of 3.4 to 18.1 per cent; a hemoglobin content of 30 to 55 per cent; 1,500,000 to 2,860,000 erythrocytes per cubic millimeter; 4,760 to 6,720 leukocytes per cubic millimeter, with neutrophils 50 to 87 per cent, lymphocytes 10 to 33 per cent and monocytes 2 to 8 per cent; serum albumin 4.5 Gm., serum globulin 1.5 Gm., blood nonprotein nitrogen 80 to 240 mg., plasma chlorides 455 mg., serum calcium 10.5 to 11.9 mg., serum phosphorus 10.5 to 16.0 mg. and serum phosphatase 7.5 to 10.7 units per hundred cubic centimeters, and carbon dioxide–combining power 49.6 volumes per cent; anorexia; dyspnea; asthenia; cough; a mass on the right coracoacromial joint; tumors on the sternoclavicular and phalangeal joints; dry, brittle, transversely ridged nails; a negative calcium balance with a low calcium intake, a positive calcium balance on a high calcium intake, and a great retention of phosphorus with a high phosphorus intake. Terminally there were pruritus, rigid arteries, epistaxis, nausea, vomiting, prostration and convulsive seizure. Autopsy disclosed: chronic hydronephrotic pyelonephritis; calcification of the heart, the systemic arteries, the lungs, the stomach, the kidneys, the periarticular structures and the dura; rickets of the long bones; otitis media on the right, due to infection with coliform organisms and pneumococci; terminal streptococcic and staphylococcic bacteremia.

The patient observed by Shelling and Remsen[45] was a youth 17 years old, who had gradual onset of deformities of all four extremities and of the chest and inability to walk for three years. Three months before admission he fractured his right femur and was confined to bed. In addition to the changes mentioned, examination showed: pallor; hard peripheral arteries; narrowing of the arterioles in the ocular fundi; a hemoglobin content of 8.6 Gm. per hundred cubic centimeters of blood; 3,200,000 erythrocytes per cubic millimeter; 10,000 leukocytes

44. Smyth, F. S., and Goldman, L.: Am. J. Dis. Child. **48**:596, 1934.

45. Shelling, D. H., and Remsen, D.: Bull. Johns Hopkins Hosp. **57**:158, 1935.

per cubic millimeter, with 53 per cent segmented neutrophils and 42 per cent lymphocytes; serum calcium 9.3 to 9.9 mg., phosphorus 7.7 to 10.5 mg., phosphatase 22.1 Bodansky units, blood nonprotein nitrogen 153 to 364 mg., blood cholesterol 208 mg. and total serum protein 4.7 to 5.9 Gm. per hundred cubic centimeters; an albumin–globulin ratio of 1.7; blood chlorides 76 to 113 milliequivalents; carbon dioxide-combining power 27 to 48 volumes per cent; normal sugar tolerance and basal metabolic rate; urine loaded with erythrocytes and leukocytes; a phenolsulfonphthalein test with excretion of less than 5 per cent in two hours; calcification of the arteries in the fingers and subcutaneous tissue, halisteresis and deformities of skeletal bones, and osteoporosis of long bones by roentgenogram; a positive test of the blood for parathyroid hormone. Terminally anorexia, drowsiness, vomiting, dehydration, acidosis, streptococcic bacteremia and stupor were observed. Post mortem chronic suppurative hydronephrotic pyelonephritis, calcification of systemic arteries, abscesses of the lungs and the liver and focal necrosis of the anterior lobe of the pituitary gland were found.

The 23 year old woman described by Magnus and Scott [46] had increasing weakness, exertional dyspnea, drowsiness, loss of weight, manual tremors, clubbed fingers, cold feet, scoliosis and brown pigmentation of the skin for ten months. In addition, examination showed: emaciation; an enlarged thyroid gland; a blood pressure of 96 systolic and 62 diastolic; hard peripheral arteries; thick subcutaneous tissue in the legs; a hemoglobin content of 43 per cent; serum calcium 11 mg. and serum sodium 319 mg. per hundred cubic centimeters; calcified arteries in the extremities and subcutaneous calcium deposits in the legs by roentgenogram. Terminally there were vomiting and coma. Chronic nephritis and calcification of the systemic arteries and of the subcutaneous tissue of the legs were observed.

Pollack and Siegal [47] reported the case of a woman 41 years old who had recurrent pain in the thighs for eighteen months, general pruritus, pigmentation of the skin, polyuria and nocturia for one year, and subcutaneous swellings, loss of weight and pain in the shoulders for six months. Examination showed: a blood pressure of 153 systolic and 76 diastolic; bilateral Babinski signs; a Chaddock sign on the left; subcutaneous nodules on the hands, the right elbow, the left infrascapular region, the left knee and the toes; a hemoglobin content of 53 per cent; erythrocytes 3,400,000 and leukocytes 4,300 per cubic millimeter; blood urea nitrogen 95 mg., serum calcium 12 to 13 mg., phosphorus 4 to 6.3 mg. and phosphatase 5 Bodansky units per hundred cubic centimeters; carbon dioxide–combining power of the blood 35 volumes per cent; albuminuria; no excretion of intramuscularly injected phenolsulfon-

46. Magnus, H. A., and Scott, R. B.: J. Path. & Bact. **42**:665, 1936.

47. Pollack, H., and Siegal, S.: J. Mt. Sinai Hosp. **2**:270, 1936.

phthalein in two hours; a basal metabolic rate of + 57 to 63 per cent; widespread calcium deposits in the shoulders, the hands, the buttocks, the pelvic blood vessels and the left foot by roentgenogram; heavy calcium deposits in a toe by biopsy; elevated serum calcium after 15 Gm. of calcium gluconate had been taken orally, indicating an increase of the blood parathyroid hormone. She received strong solution of iodine, U.S.P., and viosterol by mouth prior to a partial resection of enlarged parathyroid glands, which showed diffuse hyperplasia. Most of the thyroid gland was also resected, but it failed to reveal hyperplasia. The serum calcium fell to 10.2 mg. per hundred cubic centimeters, the blood urea nitrogen increased, oliguria developed, and high terminal fever was observed. Chronic nephritis, calcification of the lungs and the kidneys, healed tuberculosis of the lungs and the bronchial lymph nodes, two small pheochromocytomas of the adrenal medulla and cholelithiasis were demonstrated.

Price and Davie [48] recorded the case of a youth 14 years old who was deaf and who had suffered from polydipsia and polyuria for eleven years and an increasing deformity of the lower extremities with limitation of activity for six years. He exhibited stunting of stature, coarse, sparse hair, a depressed nose, large maxillas, a receded mandible, small scrotal testes and infantile secondary sex characteristics. The blood pressure was 120 systolic and 75 diastolic. There were flexion deformities of the hips, lumbar lordosis, waddling gait and genu valgum. The skeletal bones showed osteoporosis and enlarged metaphyses; the femurs, the tibias and the fibulas, bowing, and the skull a thick, rarefied, honeycombed appearance, by roentgenogram. There was slight albuminuria, with 0.85 per cent urea concentration in three hours. Blood urea was 39.7 to 318 mg., serum calcium 12.5 to 13.6 mg., serum phosphorus 5 to 6.5 mg. and serum phosphatase 51.1 to 59.6 units per hundred cubic centimeters. Terminally twitchings, convulsions and coma were observed. Chronic nephritis, calcification of a kidney and rickets of a femur were also present.

Castleman and Mallory [49] described a man 45 years old who had scarlet fever thirty-five years before, a diagnosis of Bright's disease twenty years before, and cutaneous pruritus, nocturia and swollen fingers for thirty months. Examination showed: a blood pressure of 165 systolic and 90 diastolic; precordial systolic and diastolic murmurs; firm, tortuous peripheral arteries; cystic swelling of the right forefinger; a hemoglobin content of 65 per cent; erythrocytes 3,800,000 and leukocytes 12,000 per cubic millimeter, with segmented neutrophils 63 per cent; blood nonprotein nitrogen 130 mg., serum calcium 10.1 mg.,

48. Price, N. L., and Davie, T. B.: Brit. J. Surg. **24**:548, 1937.

49. Castleman, B., and Mallory, T. B.: New England J. Med. **214**:320, 1936; Am. J. Path. **13**:553, 1937.

phosphorus 7.9 mg., phosphatase 9.4 Bodansky units, serum protein 4.9 Gm. and blood uric acid 4.4 mg. per hundred cubic centimeters; blood chlorides 107 milliequivalents; carbon dioxide-combining power of blood 37.8 volumes per cent.; moderate albuminuria; a phenolsulfonphthalein test with excretion of less than 15 per cent in one hour; masses of calcium around the finger, acromioclavicular and elbow joints, calcified blood vessels, and a skull marked by decalcified areas by roentgenogram; small kidneys by pyelogram. There were diarrhea and epistaxis, and terminally, severe pain in the chest, bundle branch block and blood pressure of 60 systolic and 50 diastolic. At autopsy chronic glomerulonephritis, calcification of systemic arteries and periarticular structures, coronary thrombosis and occlusion, and rheumatic aortic and mitral valvulitis with mitral stenosis were demonstrated.

Pons and Pappenheimer [50] reported the case of a 33 year old man who had gain of weight, polyphagia, polydipsia and polyuria for eleven years, albuminuria for nine years, morning nausea and occasional vomiting for twenty-eight months, and high blood pressure, easy fatigue and palpitation for eight months. The hemoglobin content was 62 per cent; the erythrocyte count, 2,690,000; the leukocyte count, 3,600, with 65 per cent segmented neutrophils and 40.5 per cent lymphocytes; the blood showed nonprotein nitrogen 100 to 134 mg., uric acid 5.7 to 8.7 mg., urea nitrogen 38.7 to 62.8 mg. and dextrose 142 mg. per hundred cubic centimeters in the two months before admission, when his basal metabolic rate was —22 per cent and he was afflicted with weakness, vertigo, vomiting and diarrhea. He showed pallor and had an enlarged heart; his blood pressure was 132 systolic and 92 diastolic; there was adiposity of the face, neck and trunk, and purple striae on the abdomen and thighs. Osteoporosis of the calvarium was revealed by roentgenogram. Terminally he suffered from dysphagia, dry cough, insomnia, congested lungs, tremors of the extremities and general aching. Autopsy disclosed: chronic nephritis; calcification of the heart, the systemic arteries, the lungs and the stomach; osteitis fibrosa cystica of the skull and the vertebrae; cardiac cirrhosis of the liver; hemosiderosis of the spleen.

These authors also outlined the case of a man 23 years old, who had renal symptoms for several years before autopsy disclosed subacute glomerulonephritis, metastatic calcification of the lungs and the kidneys, osteitis fibrosa in the bones, and parathyroid glands weighing 2.67 Gm. The renal calcium of this patient was 1,300 mg. per hundred grams of wet tissue, compared with a normal of 11 mg. per hundred grams.

The case of Brown and Ginsberg [51] was that of a woman 55 years old who had perennial albuminuria after scarlet fever forty-six years

50. Pons, J. A., and Pappenheimer, A. W.: Puerto Rico J. Trop. Med. **13**:115, 1938.

51. Brown, C. L., and Ginsberg, I. W.: Arch. Path. **30**:108. 1940.

before. For fourteen months she experienced weakness, gain in weight, easy fatigue, exertional dyspnea and ulcers of the legs. She displayed pallor, a 2 cm. mass on the right side of the mandible, and a deformity of the chest. Examination showed: a blood pressure of 186 systolic and 94 diastolic; a systolic murmur and thrill; rigid peripheral arteries; slight albuminuria; less than 5 per cent excretion of intramuscularly injected phenolsulfonphthalein in two hours; 12 per cent urea clearance; a bleeding time of ninety seconds; a clotting time of five minutes; blood erythrocytes 2,460,000 per cubic millimeter, leukocytes 11,400 per cubic millimeter, 64 per cent of which were segmented neutrophils, platelets 195,000 per cubic millimeter, blood urea nitrogen 68 mg., blood creatinine 2.3 mg., blood sugar 71 mg., serum cholesterol 182 mg., serum calcium 10.8 to 12.5 mg., serum phosphorus 5.2 to 7.2 mg., serum phosphatase 11.6 Bodansky units and serum protein 4.7 Gm. per hundred cubic centimeters; carbon dioxide-combining power of plasma 30 volumes per cent; halisteresis of cranial and other skeletal bones, a cystic area in the mandible, calcification of arteries, trachea and bronchi, thoracic kyphos, pelvic deformity and periostitis of the femurs, by roentgenogram; a negative calcium balance in forty-eight hours; a basal metabolic rate of + 34 to 49 per cent. Terminally there was left ventricular failure. For at least five months before death the patient took a high calcium, low phosphorus diet and 3,600 units of vitamin D daily. Chronic glomerulonephritis, calcification of the heart and systemic arteries and pulmonary edema were demonstrated post mortem.

Herbert, Miller and Richardson [52] described a 40 year old woman who had suffered from weakness, polyuria, nocturia and intermittent vomiting for four years after nephritis had been diagnosed in the last trimester of a pregnancy which terminated prematurely. For one year she had pains in the knee, ankle, wrist, elbow and spinal joints. For six months she had headaches, dyspnea, easy fatigue, edema of the ankles and pallor. Her skin was lemon yellow. Examination showed: a blood pressure of 180 systolic and 110 diastolic; a systolic thrill and murmur; rigid peripheral arteries; albuminuria; a hemoglobin content of 38 per cent; erythrocytes 1,940,000 and leukocytes 7,400 per cubic millimeter; blood urea 300 mg., serum calcium 11.7 mg., serum phosphorus 7.8 mg. and serum phosphatase 32.4 Jenner-Kay units per hundred cubic centimeters; calcification of arteries and calcific deposits around the acromioclavicular and phalangeal joints, by roentgenogram; no urinary excretion of a dye given intravenously. Terminally there was a downhill course with uremia. Autopsy disclosed: chronic glomerulonephritis; calcification of the heart, the systemic arteries and the periarticular structures; osteitis fibrosa cystica of the vertebrae, the clavicles, the fingers and the ribs.

52. Herbert, F. K.; Miller, H. G., and Richardson, G. O.: J. Path. & Bact. 53:161, 1941.

The first case described by Andersen and Schlesinger [53] concerned a boy 6 months old who had twitching of the extremities for four days before admission. Examination showed: muscle fibrillations in the extremities; a bulging left ear drum; a congested pharynx; a nasal discharge; a systolic murmur; a palpable liver; a hemoglobin content of 7.3 to 11 Gm. per hundred cubic centimeters; erythrocytes 1,900,000 to 3,570,000 per cubic millimeter; leukocytes 7,000 to 35,000 per cubic millimeter, with 52 per cent segmented neutrophils, 39 per cent lymphocytes, 6 per cent eosinophils and 2 per cent monocytes; serum calcium 7.4 to 11 mg., phosphorus 5.2 to 12.3 mg., serum phosphatase 6 to 7.3 Bodansky units, blood nonprotein nitrogen 45 to 122 mg., blood sugar 107 mg., blood urea nitrogen 56.7 to 71.7 mg., blood creatinine 2.3 mg., blood cholesterol 210 mg., serum albumin 3.8 to 4.4 Gm. and serum globulin 2.2 to 2.9 mg. per hundred cubic centimeters; blood chlorides 85.7 to 108.8 milliequivalents; carbon dioxide of blood 8.5 to 38.5 milliequivalents; slight albuminuria; Bacillus lactis aerogenes and Streptococcus viridans in the urine; puriform fluid with left myringotomy; low grade fever; episodes of vomiting; calcified blood vessels in the extremities and osteoporosis of the ends of the long bones by roentgenogram. Terminally he presented craniotabes, hard peripheral arteries, diffuse precordial systolic murmur and high fever. For one month before admission he received 500 units of vitamin D_3 as percomorph liver oil daily. For the next five months he was given 170,000 units of vitamin D_3 (same preparation) and vitamin D_2 (Drisdol). Oral alkalis were administered to control acidosis. Autopsy disclosed: chronic hydronephrotic pyelonephritis; calcification of the heart and the systemic arteries; early renal rickets in the ribs, the vertebrae and a humerus; lobular pneumonia; hemosiderosis of the spleen and the liver.

The second case of Andersen and Schlesinger [53] was that of a 6 month old boy who was admitted with a history of convulsions of one day's duration. He showed a diffuse papular cutaneous rash, signs of tetany and a round mass in the left flank. The hemoglobin content was 6.6 to 12 Gm. per hundred cubic centimeters; the erythrocyte count was 3,410,000 and the leukocyte count was 34,800, per cubic millimeter, with 83 per cent segmented neutrophils. Serum calcium was 6.5 to 9 mg., serum phosphorus 6.4 to 11.3 mg., serum phosphatase 11.4 Bodansky units, blood nonprotein nitrogen 68.4 to 125 mg., blood urea nitrogen 96.1 mg., serum albumin 4.4 Gm., serum globulin 3.1 Gm. and blood cholesterol 177 mg. per hundred cubic centimeters; the carbon dioxide–combining power of the blood was 16 to 41.4 volumes per cent. There was albuminuria, and Staphylococcus aureus and Staph. albus were present in the urine. Halisteresis of the long bones was shown

53. Andersen, D. H., and Schlesinger, E. R.: Am. J. Dis. Child. **63**:102, 1942.

by roentgenogram. Terminally, there was necrosis of the left buttock at the site of injections of calcium gluconate, and the blood pressure was 136 to 150 systolic and 90 diastolic. Calcium chloride, calcium gluconate and 300,000 units of vitamin D as 7-dehydrotachysterol alleviated the tetanus on admission. The patient also received 15 drops of a vitamin D preparation for two months of his hospital stay and calcium gluconate and sodium lactate orally for the last three weeks of life. Autopsy disclosed: chronic hydronephrotic pyelonephritis of the right kidney; a polycystic left kidney; calcification of the heart, the systemic arteries and the right kidney; pulmonary edema; hemosiderosis of the spleen.

Summary.—Of the 23 patients with chronic renal disease and metastatic calcification, the sex was male in 14 and female in 9. Their ages were between 6 months and 55 years, the disease occurring in 17 before the age of 40 and in only 1 who was over 50 years of age. Four infants were affected. The urine showed varying degrees of albuminuria,[54] with specific gravity ranging from 1.000 to 1.017, but generally being less than 1.010. When recorded,[55] the number of leukocytes and erythrocytes found in the urine was variable. The excretion of phenolsulfonphthalein [56] ranged from none to less than 10 per cent in two hours. Urea clearance [57] varied from less than 1 to 18 per cent. The level of hemoglobin and of blood erythrocytes was moderately to greatly depressed in several patients.[58] The level of serum calcium was depressed (6.7 mg. per hundred cubic centimeters),[43] depressed or normal (7.4 to 11.0 mg. and 6.5 to 90 mg.),[53] normal (9.3 to 11.0 mg.)[59] or elevated (11.7 to 13.6 mg.).[60] Serum inorganic phosphorus was elevated (5 to 16 mg. per hundred cubic

54. Butler.[39] Lightwood.[41] Albright and others.[42] Platt and Owen.[43] Smyth and Goldman.[44] Pollack and Siegal.[47] Price and Davie.[48] Castleman and Mallory.[49] Brown and Ginsburg.[51] Herbert and others.[52] Andersen and Schlesinger.[53]

55. Butler.[39] Lightwood.[41] Albright and others.[42] Smyth and Goldman.[44] Shelling and Remsen.[45] Pollack and Siegal.[47] Castleman and Mallory.[49] Herbert and others.[52] Andersen and Schlesinger.[53]

56. Butler.[39] Smyth and Goldman.[44] Shelling and Remsen.[45] Pollack and Siegal.[47] Brown and Ginsburg.[51]

57. Lightwood.[41] Smyth and Goldman.[44] Price and Davie.[48] Brown and Ginsburg.[51]

58. Albright and others.[42] Platt and Owen.[43] Smyth and Goldman.[44] Shelling and Remsen.[45] Magnus and Scott.[46] Pollack and Siegal.[47] Castleman and Mallory.[49] Pons and Pappenheimer.[50] Brown and Ginsberg.[51] Herbert and others.[52] Andersen and Schlesinger.[53]

59. Lightwood.[41] Shelling and Remsen.[45] Magnus and Scott.[46] Castleman and Mallory.[49]

60. Hubbard and Wentworth.[38] Smyth and Goldman.[44] Pollack and Siegal.[47] Price and Davie.[48] Brown and Ginsburg.[51] Herbert and others.[52]

centimeters) in 11 patients,[61] although 2 also showed normal values on occasion. The alkaline phosphatase of the serum was slightly to moderately raised in 7 patients[62] and normal in 2.[53] The blood nonprotein nitrogen (45 to 364 mg. per hundred cubic centimeters)[63] and urea (143 to 560 mg. per hundred cubic centimeters)[64] or urea nitrogen (38.7 to 205 mg. per hundred cubic centimeters)[65] were increased moderately to greatly in all cases in which the values were recorded. The carbon dioxide–combining power of the blood was slightly to severely lowered (16 to 49.6 volumes per cent) in 7 patients.[66] The serum proteins (4.7 to 7.5 Gm. per hundred cubic centimeters) were somewhat lowered or normal. Blood cholesterol (177 to 210 mg. per hundred cubic centimeters) was slightly to moderately elevated in 6 patients.[67] Blood chlorides were depressed or normal in 4 patients.[68]

The types of nephropathy found associated with metastatic calcification in the 23 cases of renal disease included chronic nephritis in 9, chronic hydronephrotic pyelonephritis in 4, chronic glomerulonephritis and chronic interstitial nephritis in 3 each, chronic suppurative hydronephrotic pyelonephritis (pyonephrosis) in 2 and subacute glomerulonephritis and active degenerative nephritis in 1 each. In 1 case,[40] amyloidosis involved the right kidney, and chronic suppurative hydronephrotic pyelonephritis affected the left kidney. In the second case of Andersen and Schlesinger,[53] chronic hydronephrotic pyelonephritis was present in the right kidney, and a polycystic left kidney was found. In the other 21 cases the lesion was bilateral as indicated.

The parathyroid glands of 15 patients were described. In 11 instances these glands were enlarged and showed diffuse hyperplasia of chief cells, characteristic of chronic renal insufficiency.[49] Five glands were found involved by Smyth and Goldman[44]; 4 each by Shelling and Remsen,[45] Price and Davie,[48] Castleman and Mallory,[49] Pons and Pappenheimer[50]

---

61. Lightwood.[41] Platt and Owen.[43] Smyth and Goldman.[44] Shelling and Remsen.[45] Pollack and Siegal.[47] Price and Davie.[48] Castleman and Mallory.[49] Brown and Ginsburg.[51] Herbert and others.[52] Andersen and Schlesinger.[53]

62. Smyth and Goldman.[44] Shelling and Remsen.[45] Pollack and Siegal.[47] Price and Davie.[48] Castleman and Mallory.[49] Brown and Ginsberg.[51] Herbert and others.[52]

63. Platt and Owen.[43] Smyth and Goldman.[44] Shelling and Remsen.[45] Castleman and Mallory.[49] Pons and Pappenheimer.[50] Andersen and Schlesinger.[53]

64. Butler.[39] Lightwood.[41] Price and Davie.[48] Herbert and others.[52]

65. Albright and others.[42] Platt and Owen.[43] Pollack and Siegal.[47] Pons and Pappenheimer.[50] Brown and Ginsberg.[51] Andersen and Schlesinger.[53]

66. Smyth and Goldman.[44] Shelling and Remsen.[45] Pollack and Siegal.[47] Castleman and Mallory.[49] Brown and Ginsberg.[51] Andersen and Schlesinger.[53]

67. Lightwood.[41] Platt and Owen.[43] Shelling and Remsen.[45] Brown and Ginsberg.[51] Andersen and Schlesinger.[53]

68. Smyth and Goldman.[44] Shelling and Remsen.[45] Castleman and Mallory.[49] Andersen and Schlesinger.[53]

(first case), Herbert, Miller and Richardson [52] and Andersen and Schlesinger [53] (first case); 3, by Magnus and Scott [46]; 2, by Hubbard and Wentworth.[58] Pollack and Siegel [47] did not specify the number of enlarged glands present. In the second case of Andersen and Schlesinger [53] the left lower gland was affected. Lightwood [41] described one normal gland in his case. Albright, Baird, Cope and Bloomberg [42] mentioned enlarged lower parathyroid glands in their case, and Platt and Owen [43] stated that their patient's glands were not enlarged, but in neither instance was a microscopic description given. In the report of their second case Pons and Pappenheimer [60] did not mention the number or the histologic character of the enlarged parathyroid glands.

In the 13 cases in which the bones (including the ribs, the vertebrae, the long bones and the skull bones) were examined, osteitis fibrosa cystica was the lesion in 7, rickets in 4 and osteoporosis in 2.

Also noteworthy in 5 cases [69] was the use of vitamin D.

Calcium deposits were observed in the lungs in 12 cases, most often in the walls of the alveoli and in the intima and the media of arteries and veins, frequently in relation to the elastic tissue in these structures. Bronchi, bronchioles, alveolar ducts and lumens, capillaries and stroma were less commonly affected.

In 10 cases significant calcification was found in the kidneys, most often in the cells and lumens of convoluted and collecting tubules and in the stroma. Glomeruli and the basement membranes of tubules were less frequently involved. In only 1 instance was deposition of calcium described [44] in the smaller arteries and arterioles of the kidneys. In no case was calcification of renal veins shown.

In 11 cases calcification of the heart was described. The muscle fibers, chiefly those of the left ventricle, the stroma of the myocardium and the endocardium of the left auricle and ventricle were especially affected. Small arteries, capillaries, the mitral valve and the epicardium were involved to a minor degree.

In 15 cases the systemic arteries showed calcific changes. In addition to the aorta, the coronary, mesenteric, splenic, hepatic, renal, gastric, pancreatic, adrenal, uterine, vesical, hypogastric, thyroid, intercostal, vertebral, subclavian, axillary, brachial, iliac, femoral, popliteal, tibial and pedal arteries were affected. The calcium salts were found chiefly in the internal elastic lamella of the intima and in the media, often in relation to elastic fibrils.

In 3 cases, calcification involved the stomach; it was abundant in the interglandular stroma, but was also found in the basement membranes of the glands.

---

69. Lightwood.[41] Pollack and Siegal.[47] Brown and Ginsberg.[51] Andersen and Schlesinger.[53]

Miscellaneous sites of calcification included periarticular structures,[70] dura,[71] tentorium,[41] trachea,[41] parietal pleura,[41] jejunum,[38] greater omentum,[38] splenic veins,[37] skin [43] and subcutaneous tissue.[40]

Schmidt [37] found calcium phosphate and little calcium carbonate in the heart of his patient. As the oxide, calcium amounted to 2.3 per cent in the left ventricle and 0.6 per cent in the right ventricle of this patient by quantitative analysis. In their case, Smyth and Goldman [44] found calcium phosphate in the periarticular deposits and 20.92 mg. of calcium and 11.54 mg. of phosphorus by dry weight in the long bones. The calcium content of the kidneys of the second patient of Pons and Pappenheimer [50] was 1,300 mg. per hundred grams of wet tissue, compared with a normal of 11 mg. per hundred grams.

METASTATIC CALCIFICATION AND PRIMARY NEOPLASMS OF THE PARATHYROID GLANDS

The first case reported by Meyer [72] concerned a married woman 43 years old, who had a therapeutic abortion for "renal inflammation" ten years before, a stillbirth nine years before, two spontaneous abortions eight years before and treatment for renal calculi for eight years. On the day of admission she fractured her left femur, which was amputated proximal to the cystic fracture site several weeks later. Osteitis fibrosa cystica of the bones of the lower extremities, absence of the left lower extremity, fracture of the right femur, calcification of the kidneys, a polyp of the uterine cervix and a myxoma of the right fallopian tube were demonstrated at autopsy. Although the organs of the neck were not examined, the patient presumably had a parathyroid neoplasm, for the changes in the bones and the kidneys were like those to be described in the summaries of other cases in this section.

Meyer's second patient was a man 36 years old, who had ataxia, severe pain in the extremities, atrophy of the thigh muscles, edema of the legs, hydrarthrosis of the left knee and polydipsia when he was first hospitalized. During the next two years he became gradually more disabled. On the day of his second admission he sustained fractures of the femurs, which were splinted. He later displayed general irritability, pallor, the typical changes of bone softening, fractures of the humeri and terminal bronchopneumonia. Autopsy disclosed: chief cell adenoma of the right inferior parathyroid gland; osteitis fibrosa cystica of the skull, vertebrae, ribs and long bones; multiple cysts and fractures of the long bones; calcification of the kidneys; right-sided bronchopneumonia; fatty metamorphosis of the liver.

---

70. Hubbard and Wentworth.[38] Smyth and Goldman.[44] Castleman and Mallory.[40] Herbert and others.[52]

71. Lightwood.[41] Smyth and Goldman.[44]

72. Meyer, O.: Frankfurt. Ztschr. f. Path. **20**:115, 1917.

Dawson and Struthers [73] reported the case of a man 49 years old, who fractured the lower third of his left humerus four months before, for which routine treatment was instituted. He displayed wasting and limitation of motion of the left upper extremity. Decalcification of the humeral fragments and an enlarged, decalcified skull were shown by roentgenogram. The urine revealed transient slight albuminuria, and biopsy of the left humerus revealed changes consistent with osteitis fibrosa cystica. About fourteen weeks later he sustained a second fracture of the left humerus proximal to the first fracture, as shown by roentgenogram; it was healing slowly. Over five months after the second fracture he was seized with a "heart attack" and died in less than a day. Autopsy disclosed: bronchopneumonia; a transitional chief cell adenoma of the left inferior parathyroid gland; osteitis fibrosa cystica of the left humerus and femur, the ribs and the skull; calcification of the heart, the lungs, the systemic arteries, the stomach, the kidneys, the spleen, the liver, the skeletal muscle, the dura and the pituitary and pineal glands.

Hoffheinz [74] described a 42 year old woman whose right arm had been fractured two years before and left knee joint one year before. Severe pains in the thighs and inability to walk confined her to bed for four months. Examination showed: atrophic thigh muscles; contractures of the adductor muscles of the thighs; limited motion of the joints; severe halisteresis of the skeleton, thick skull and long bones, skeletal cysts and two renal calculi on the right side by roentgenogram; a blood pressure of 85 systolic and 55 diastolic; blood nonprotein nitrogen 221 mg. per hundred cubic centimeters. Terminally there was uremic coma. Autopsy disclosed: diffuse hyperplasia of wasserhelle cells of four enlarged parathyroid glands; osteitis fibrosa cystica of skull, vertebrae and femurs; a fracture of the neck of the right femur; a cyst of the shaft of the left femur; chronic hemorrhagic suppurative cystoureteropyelonephritis; calcification of the kidneys, a coronary artery, the lungs and the stomach; renal calculi; fatty metamorphosis of the liver; nodular cortical hyperplasia of the adrenal glands.

Penecke [75] examined 2 patients at autopsy. The first was a man 38 years old, with a 16 Gm. transitional oxyphilic cell adenoma of the left inferior parathyroid gland, osteitis fibrosa cystica of the bones, and calcification of the contracted kidneys, the heart, the systemic arteries, the spleen, the tongue, the thyroid gland, the wall of the parathyroid neoplasm and the skin. The second was a woman 59 years old, with a 5 Gm. chief cell adenoma of the right inferior parathyroid gland, osteitis fibrosa cystica of the bones and calcification of the kidneys.

73. Dawson, J. W., and Struthers, J. W.: Edinburgh M. J. **30**:421, 1923.
74. Hoffheinz: Virchows Arch. f. path. Anat. **256**:705, 1925.
75. Penecke: Centralbl. f. allg. Path. u. path. Anat. **37**:535, 1926.

Fontana [76] reported the case of a woman 26 years old, who had severe headache, loss of weight, fatigue and intermittent fever with vomiting and delirium for nine years before admission. In the year before she died she had albuminuria, edema of the ankles, vomiting, exertional dyspnea, weakness, fever, painful, fluctuant swellings adjacent to the large joints of the trunk and extremities and, terminally anasarca, hard peripheral arteries, increased dyspnea, precordial pain, diffuse pulmonary congestion, prostration and subnormal temperature. At autopsy the following conditions were demonstrated: a chief cell adenoma of the left superior parathyroid gland; osteomalacia of the skull, the sternum and the ribs; calcification of the kidneys, the heart, the systemic arteries, the lungs, the bursas and the dura; arteriolosclerosis of the spleen; fatty metamorphosis of the liver; atrophy of the ovaries; partial atrophy of the thyroid gland, the anterior lobe of the pituitary gland and the cortices of the adrenal glands.

Ask-Upmark [77] reported the case of a man 46 years old, who suffered with loss of weight, pain in the feet and the joints of the hip and the knee for nine months, insomnia, anorexia, and pain in the head, the spine and the extremities for three months, so that he became bedridden, and cough, vomiting and occasional hemoptysis for two weeks. Examination showed: a systolic blood pressure of 175; emaciation; atrophic pharyngitis; dental caries; hemorrhagic gingivitis; pain on motion of the left hip and knee; a hemoglobin content of 76 per cent; erythrocytes 3,980,000 per cubic millimeter; leukocytes 14,200 per cubic millimeter, with 55 per cent neutrophils, 32 per cent lymphocytes and 13 per cent monocytes; serum calcium 10.1 mg., and blood nonprotein nitrogen 50 mg. per hundred cubic centimeters; slight albuminuria; lumbar lordosis, mottled lumbar vertebrae, halisteresis of the sacrum, the pelvic bones, the femurs, the scapulas, the humeri, the clavicles and the ribs and destruction of the cortex of the left femur, by roentgenogram; spontaneous fractures of the humeri and a femur; hard peripheral arteries. Terminally there were fecal and urinary incontinence, decubital ulcers and intermittent fever. Autopsy showed: a chief cell adenoma of a left parathyroid gland; osteitis fibrosa cystica of the skull, the vertebrae, the pelvic bones and the femurs; fractures of the humeri and the femurs; calcification of the kidneys and the coronary arteries; bronchopneumonia.

A woman 57 years old, described by Bergstrand,[78] had general weakness, severe constipation, piercing left-sided headaches localizing in the left ear, a systolic blood pressure of 190 to 205, and loss of 15 Kg. for seven months, and abdominal distention and severe exertional palpitation and dyspnea for two months, so that she was forced to go to bed. She

76. Fontana, A.: Endocrinol. e pat. costit. **4**:401, 1929.
77. Ask-Upmark, E.: Acta med. Scandinav. **74**:284, 1930.
78. Bergstrand, H.: Acta med. Scandinav. **76**:128. 1931.

exhibited pallor, nervousness, a fine tremor of the fingers and a goiter of moderate size. Examination showed: blood pressure varying from 140 systolic and 95 diastolic to 180 systolic and 110 diastolic; a soft blowing systolic murmur over both the heart and the root of the aorta; a uterine tumor; a hemoglobin content of 95 per cent; erythrocytes 3,900,000 per cubic millimeter; leukocytes 4,800 per cubic millimeter, with segmented neutrophils 52 per cent, lymphocytes 36 per cent and monocytes 10 per cent; blood nonprotein nitrogen 40 mg. per hundred cubic centimeters; a basal metabolic rate of +14 to 41 per cent; total gastric acidity 22. Two years after discharge she had essentially the same symptoms and signs except for 35 per cent segmented neutrophils and 61 per cent lymphocytes in the blood, slight albuminuria and urinary specific gravity of 1.005 to 1.008, a total gastric acidity of 7, and widening of the right side of the superior mediastinum, seen by roentgenogram. Terminally a brown color of the skin developed, with fever, nausea, vomiting, and a painful mass in the right upper abdominal quadrant. At autopsy the following conditions were found: diffuse follicular hyperplasia of four enlarged parathyroid glands; osteitis fibrosa cystica of the skull, the lumbar vertebrae and the femurs; calcification of the kidneys, the renal and splenic arteries and the liver; cardiac hypertrophy; acute and chronic cholecystitis; cholelithiasis; mild diffuse hyperplasia of the thyroid gland; a leiomyoma of the uterus.

Paul [79] reported the case of a 56 year old man, who had anorexia, weakness and loss of weight for two years, polyuria and nocturia for one year, vomiting for one month and severe headache for one week. His blood pressure was 184 systolic and 100 diastolic, and he had albuminuria with many hyaline casts, frequent emesis, rapid pulse, an apical diastolic murmur and terminal acute cardiac failure. Autopsy disclosed: diffuse hyperplasia of wasserhelle cells of the two enlarged superior parathyroid glands; osteitis fibrosa cystica of the skull, the vertebrae, the ribs, the pelvic bones and the femurs; fractures of four ribs; cysts of the femurs; calcification of the kidneys, the thyroid arteries, the splenic arterioles and the liver; pulmonary edema; suppurative bronchitis; nodular cortical hyperplasia of the adrenal glands.

Hand [80] recorded the case of a man 39 years old, who had not felt well for nine years before admission. He had nocturia for four months, an attack of rheumatism two months before, penile pain, urgency and frequency for one month and milky penile discharge and loss of 30 pounds (13.6 Kg.) for two weeks. He displayed emaciation, pallor and a dry skin. Examination showed: a small thyroid gland; a pulse rate of 140 beats per minute; a blood pressure of 130 systolic and 70 diastolic; normal heart and lungs; a palpable right kidney; a small,

79. Paul, F.: Beitr. z. path. Anat. u. z. allg. Path. **87**:503, 1931.
80. Hand, J. R.: S. Clin. North America **13**:1365, 1933.

tender prostate; normal testes; albuminuria; erythrocytes, leukocytes and gram-negative intracellular diplococci in the urine; a hemoglobin content of 94 per cent; erythrocytes 4,990,000 per cubic millimeter; leukocytes 10,000 per cubic millimeter with neutrophils 49 per cent and lymphocytes 47 per cent; a sedimentation rate of 18 mm. in forty-five minutes; serum calcium 14.6 mg. and blood urea 46 mg. per hundred cubic centimeters; a phenolsulfonphthalein test with excretion of 56 per cent in two hours; a dilated colon and lumbar scoliosis by roentgenogram; severe cystitis by cystoscopy. He received a high caloric, high vitamin diet, a 5 per cent solution of dextrose intravenously, 8 drachms (31 Gm.) of citrocarbonate, 3 drachms (11.65 Gm.) of calcium lactate and 10 drops of viosterol daily. Between seven weeks and the time of his death, sixteen weeks later, he suffered successively from urinary frequency and loss of strength, scleritis, conjunctivitis, left corneal ulcer, low grade fever, tachycardia, pigmentation of the skin, atrophy of muscles, bloating and, terminally, from expectoration of much purulent sputum. Autopsy disclosed: a chief cell adenoma of the left inferior parathyroid gland; general osteoporosis of the skull and the vertebrae; subacute interstitial nephritis; multiple renal abscesses; calcification of the kidneys, the heart, and the lungs; hypertrophy of the urinary bladder; cystitis cystica; chronic prostatitis.

Khurgina [81] reported the case of a woman 34 years old, who showed at autopsy severe gastritis and abdominal dermatitis, an adenoma of a parathyroid gland, osteosclerosis of the skeleton and calcification of the kidneys, the heart, the lungs and the stomach.

Laubmann [82] described a case in which clinically there were observed osteitis fibrosa cystica, cutaneous calcium deposits, rigid arteries in the lower extremities, increased serum calcium, leukocytosis and terminal anemia. Autopsy revealed an adenoma of a parathyroid gland, osteitis fibrosa cystica of the bones, and calcium deposits in the heart, the blood vessels, the lungs, the spleen and the skin.

The 49 year old woman described by Hanes [83] had diagnoses of renal calcification and hydronephrosis of the right kidney on the basis of urinary, hematologic and roentgenologic studies five years before admission to the hospital. She had right infrascapular pain for twenty-one months, pain along the anterior aspect of the right third rib for seventeen months, and weakness, loss of weight and confinement to bed for three months. Examination showed: a temperature of 38.2 C. (100.7 F.); a pulse rate of 120 and a respiratory rate of 22 per minute; a blood pressure of 148 systolic and 90 diastolic; emaciation; a 2 cm. nodule at the lower pole of the left lobe of the thyroid gland; slight

81. Khurgina, P. A.: Klin. med. **11**:1238, 1933.
82. Laubmann, W.: Verhandl. d. deutsch. path. Gesellsch. **27**:229, 1934.
83. Hanes, F. M.: Am. J. M. Sc. **197**:85, 1939.

albuminuria; a hemoglobin content of 10 Gm. per hundred cubic centimeters; erythrocytes 3,500,000 and leukocytes 6,200 per cubic millimeter; serum calcium 20 to 22 mg., serum phosphorus 4.8 mg., phosphatase 23 Bodansky units, plasma nonprotein nitrogen 58 mg., and total serum protein 6.2 Gm. per hundred cubic centimeters; kidneys mottled by calcium deposits and bones decalcified, by roentgenogram; a phenolsulfonphthalein test with excretion of 40 per cent in ninety minutes. Terminally there were fever, tachycardia, weakness, nervousness, hoarseness, generalized pain, dyspnea and cyanosis. Autopsy disclosed: an adenoma of the left inferior parathyroid gland, chronic tubular and glomerular nephritis, and calcification of the kidneys, the heart, the arteries, the lungs and the stomach.

Oliver's [84] first case concerned a woman aged 57, who lost weight and strength for six months, had nausea, anorexia, vomiting and constipation for two weeks and intermittent drowsiness for two days before admission to the hospital. Examination showed: a temperature of 97 F.; a pulse rate of 88 and a respiratory rate of 15 per minute; a blood pressure of 160 systolic and 100 diastolic; a dry, brown tongue; a normal heart; emphysematous lungs; a palpable, tender left kidney; increased reflexes on the right side; albuminuria; leukocytes and hyaline casts in the urine; blood urea 76 to 136 mg. and serum calcium 17.4 mg. per hundred cubic centimeters; carbon dioxide–combining power 75.8 volumes per cent. Terminally, severe stupor, a blood pressure of 70 systolic and 50 diastolic, a dry skin and elevated vital signs were observed. Necropsy revealed: a chief cell adenoma of the right inferior parathyroid gland; calcium deposits in the kidneys, the heart, the systemic arteries, the lungs, the stomach and the liver; a small meningioma in the right side of the anterior cranial fossa.

Oliver's [84] second patient was a woman of 56 years who had two attacks of vomiting, anorexia, constipation and severe thoracic and abdominal pain one year before and three months before admission to the hospital. She displayed drowsiness, frequent vomiting, pallor and a dry skin and tongue. Examination showed: a temperature of 98.4 F.; a pulse rate of 100 and a respiratory rate of 10 to 35 per minute; a blood pressure of 104 systolic and 86 diastolic; a small tumor adjacent to the left side of the thyroid gland; feeble cardiac tones; basal rales in the lungs; a tender abdomen; depressed reflexes; albuminuria; a hemoglobin content of 61 per cent; erythrocytes 4,660,000 per cubic millimeter; blood urea 176 mg. per hundred cubic centimeters, carbon dioxide–combining power 50.7 volumes per cent. Terminally she had uremia. Necropsy demonstrated a chief cell adenoma of the left inferior parathyroid gland and calcification of the kidneys, the heart, the systemic arteries, the lungs and the stomach.

84. Oliver, W. A.: Lancet **2**:240, 1939.

Smith and Cooke [85] reported the case of a woman 44 years old, who suffered from attacks of dizziness, rheumatism of one hand, limp, bowing of the thighs, severe pain in the lower extremities and constipation during the thirty months before she was admitted to the hospital. She had abdominal pain, vomiting, constipation and frequency of urination. Examination showed: albuminuria; serum calcium 23 mg. and blood urea 155 mg. per hundred cubic centimeters. Terminally there were sphincter incontinence, delirium, tachycardia, hyperpnea, nausea and abdominal distention. Autopsy disclosed: a chief cell adenoma of the right inferior parathyroid gland; osteitis fibrosa cystica of the skull, one ilium and one femur; calcification of the kidneys; pancreatic fat necrosis.

Alexander and co-workers [86] described a 29 year old woman who experienced weakness, anorexia, fatigability, loss of weight and vomiting during the twenty-one months before she was admitted to the hospital. The hemoglobin content was 12.5 Gm. per hundred cubic centimeters; the erythrocytes numbered 4,350,000 per cubic millimeter and the sedimentation rate was 62 mm. in an hour. There was tachycardia, and terminally there were abdominal pain, restlessness and cyanosis. Necropsy revealed a chief cell adenoma of the right inferior parathyroid gland, osteitis fibrosa cystica of the skull, the ribs and the vertebrae and calcification of the kidneys and the lungs.

Cope [87] reported the case of a man of 68 years who had weakness, especially of the lower extremities, and eventual confinement to bed during the eighteen months before hospitalization. Examination showed: transient albuminuria; a phenolsulfonphthalein test with excretion of 12 per cent in two hours; serum calcium 10 to 11.3 mg., serum phosphorus 4.7 to 5.6 mg., serum phosphatase 19 to 22.5 units, total serum protein 6.4 Gm., blood nonprotein nitrogen 29 mg. and blood uric acid 6.6 mg. per hundred cubic centimeters; plasma chlorides 107 to 115 milliequivalents per liter; carbon dioxide 16 to 24 milliequivalents per liter; rarefied areas in the skull, decalcified spine, pelvis and long bones, and cysts in the second lumbar vertebra and one ilium, by roentgenogram; later normal values for serum calcium, phosphorus and phosphatase. There were several episodes of hematemesis, and terminally signs of pyelonephritis. Autopsy demonstrated: an adenoma of the right superior parathyroid gland; osteitis fibrosa cystica of the skull, the vertebrae, the ribs and one ilium; calcification of the lungs; two tumors of the stomach, diagnosed leiomyoma.

Anderson [88] recorded the calcific and other pathologic changes observed only in the kidneys of a woman of 47 years who had operative

85. Smith, F. B., and Cooke, R. T.: Lancet **2**:650, 1940.

86. Alexander, H. B.; Pemberton, J. de J.; Kepler E. J., and Broders, A. C.: Am. J. Surg. **55**:157, 1944.

87. Cope, O.: Surgery **16**:273, 1944.

(Footnotes continued on next page)

removal of a transitional oxyphilic cell adenoma of the right superior parathyroid gland and died several months later.

Gissel and Bufe [89] described a 27 year old woman who clinically showed osteitis fibrosa cystica and an enlarged thyroid gland and whose serum calcium was determined to be 8.4 to 9.1 mg. per hundred cubic centimeters. Operative exploration of the neck revealed four grossly normal parathyroid glands, which were removed along with two thirds of the thyroid gland and a persistent thymus. Two of the parathyroid glands were transplanted into a rectus muscle. For twenty-two days after operation the patient received supplementary calcium and 60 cc. of dihydrotachysterol. Her blood calcium rose from 6.5 to 18 mg. per hundred cubic centimeters in this period, near the end of which weakness developed, with loss of weight, vomiting and severe headaches, and the patient died. Osteitis fibrosa cystica of the skeletal bones and calcification of the kidneys, the heart, the lungs, the liver and the pancreas were observed at autopsy. Although no histologic description of the parathyroid glands was given and no definite parathyroid neoplasm was found, the changes in the bones and the kidneys strongly indicated that such a tumor was present.

Summary.—There were 21 patients with primary parathyroid neoplasm associated with metastatic calcification. Of 20 of these the sex was male in 7 and female in 13. In 20 cases the age varied between 26 and 68 years, the disease occurring before the age of 40 in 7 and after that age in 13. The urine showed varying amounts of albumin,[90] with specific gravity ranging from 1.005 to 1.018. The numbers of leukocytes and erythrocytes in the urine varied.[91] The phenolsulfonphthalein excretion [92] varied from 12 to 56 per cent in two hours. The levels of hemoglobin and erythrocytes were moderately lowered in several patients.[93] The values of serum calcium were normal (10.0 to 11.3 mg. per hundred cubic centimeters)[94] or moderately to tremendously elevated (12 to 23 mg).[91] The values for serum inorganic phosphorus (4.7 to 5.6 mg. per hundred cubic centimeters) and phosphatase (19 to 23 Bodansky units) were recorded in only 2 cases.[95] The blood nonprotein nitrogen ranged between 29 and 221 mg. per hundred cubic centimeters

88. Anderson, W. A. D.: Endocrinology **24**:372, 1939.

89. Gissel, H., and Bufe, W.: Deutsche Ztschr. f. Chir. **256**:58, 1942.

90. Ask-Upmark.[77] Bergstrand.[78] Paul.[79] Hand.[80] Hanes.[83] Oliver.[84] Smith and Cooke.[85] Cope.[87]

91. Hand.[80] Hanes.[83] Oliver.[84] Smith and Cooke.[85]

92. Hand.[80] Hanes.[83] Cope.[87]

93. Ask-Upmark.[77] Bergstrand.[78] Paul.[79] Hand.[80] Oliver.[84] Alexander and others.[86]

94. Ask-Upmark.[77] Cope.[87]

95. Hanes.[83] Cope.[87]

in 5 patients,[96] and the blood urea between 46 and 176 mg. per hundred cubic centimeters in 4.[97] Other blood chemistry values were too fragmentary for consideration.

The neoplasm found in the parathyroid glands was chief cell adenoma in 10 cases, adenoma of unspecified type in 4, diffuse hyperplasia of wasserhelle cells in 3 and transitional oxyphilic cell adenoma in 2; in 2 cases a neoplasm was not demonstrated.

The lesion involving the bones in 17 cases was designated as osteitis fibrosa cystica in 14 cases and as osteomalacia, osteoporosis and osteosclerosis in 1 each. Of the 4 remaining cases, the bones were not examined in 2 and not described microscopically in 2. The bones examined the most frequently were the skull bones (11), the vertebrae (9), the femurs (7), the ribs (6) and the pelvic bones (5). Vitamin D was employed in the treatment of 2 patients.[98]

Calcium deposits were found in the lungs in 12 cases, frequently in alveolar walls, bronchi and capillaries and occasionally in arteries, veins and stroma.

In 19 cases calcification affected the kidneys, involving mainly the stroma and the cells and lumens of the convoluted and collecting tubules. The glomeruli, the arterioles and the arteries were fairly frequent sites of calcium deposition.

In 9 cases, calcium deposits were observed in the heart, preponderantly in the muscle fibers of the left ventricle, but were sometimes present in the left auricle, the capillaries and the stroma.

In 10 cases the systemic arteries were involved by calcific changes, including the aorta and the coronary, thyroid, adrenal, renal, hepatic, gastric, ovarian, meningeal, brachial, radial, iliac and femoral arteries. The internal elastic lamina of the intima and the elastic fibrils of the media were the sites of calcium deposits.

In 6 cases the stomach was the site of calcification, which involved the interglandular stroma, gland cells and blood vessels.

Miscellaneous deposits of calcium were observed in the liver,[99] the spleen,[100], the skin,[101] the dura,[102] the thyroid gland,[75] the bursas,[76] the pancreas [89] and the tongue.[75]

Chemical analyses of calcified tissues were lacking in all 21 cases.

---

96. Hoffheinz.[74] Ask-Upmark.[77] Bergstrand.[78] Hanes.[83] Cope.[87]

97. Hand.[80] Oliver.[84] Smith and Cooke.[85]

98. Hand.[80] Gissel and Bufe.[89]

99. Dawson and Struthers.[73] Bergstrand.[78] Paul.[79] Oliver.[84] Gissel and Bufe.[89]

100. Dawson and Struthers.[73] Penecke.[75] Laubmann.[82]

101. Penecke.[75] Laubmann.[82]

102. Dawson and Struthers.[73] Fontana.[76]

METASTATIC CALCIFICATION AND HYPERVITAMINOSIS D

A summary of the cases of metastatic calcification associated with hypervitaminosis D should be prefaced by reference to cases of bone disease,[28] chronic renal disease [69] and primary neoplasm of the parathyroid glands,[98] in which vitamin D employed in therapy may have contributed somewhat to the metastatic calcification observed in them.

A 5½ month old boy, described by Putschar,[103] took 6 drops of a proprietary preparation of irradiated ergosterol in oil daily for fourteen weeks, suffered from loss of weight and vomiting for ten weeks and showed slight albuminuria, a thickened skin and periods of low temperature for four weeks before he died with a terminal high fever. Autopsy disclosed calcification of the kidneys, chronic myocarditis, moderate fatty metamorphosis of the liver, subcutaneous lipid granuloma, an accessory spleen and emaciation. The parathyroid glands and bones were not described.

Thatcher [104] reported the case of an 18 month old boy who was afflicted by poor gain in weight and dyspepsia for thirteen months before being admitted to the hospital. Five months before, he had an attack of diarrhea. For five months he received irradiated ergosterol. Examination showed: general weakness; anorexia; pallor; loss of weight; an open anterior fontanel; a nightly temperature of 99.6 F.; moderate albuminuria and ketonuria; blood urea 90 mg. per hundred cubic centimeters; a systolic blood pressure of 78; apathy; hyperpnea. The course was downhill in the last twelve days of life. Autopsy demonstrated calcification of the kidneys and severe fatty metamorphosis of the liver. The ribs were not remarkable. The parathyroid glands were not examined.

Thatcher's [105] second patient was a boy 11½ months old, who showed pallor, anorexia, nervousness and constipation for four months, during which he received cod liver oil, a little of a proprietary preparation of malt extract and bone marrow and two ultraviolet ray treatments. Examination revealed loss of weight, pallor, weakness, prostration, a low temperature, atrophy of muscles, slight albuminuria. Terminally there were severe pyrexia and convulsions. Calcification of the kidneys, slight fatty change of the liver, normal bones and one intact parathyroid gland were observed at necropsy.

The boy observed by Ross and Williams [106] was one of premature twins and 8 to 14 months old. He received irradiated ergosterol for several months, during which he had anorexia, loss of weight and vomiting; he died suddenly. Autopsy disclosed asphyxia due to aspira-

103. Putschar, W.: Ztschr. f. Kinderh. **48**:269, 1929.
104. Thatcher, L.: Edinburgh M. J. **38**:457, 1931.
105. Thatcher, L.: Lancet **1**:20, 1936.
106. Ross, S. G., and Williams, W. E.: Am. J. Dis. Child. **58**:1142, 1939.

tion of food, bronchopneumonia and calcification of the kidneys, the heart, the arteries, the lungs and the stomach. The bones and the parathyroid glands were not described.

Gissel and Bufe [89] described experiments with 4 infants afflicted with meningomyelocele and hydrocephalus. The first infant received over 900 cc. of dihydrotachysterol in one hundred and twenty-eight days of life. The blood calcium rose from 6.6 mg. per hundred cubic centimeters of serum on the thirty-seventh day to 9.4 mg. on the sixty-ninth day. Calcification of the kidneys, ascending meningitis and pyocephalus were observed at necropsy. The second infant was given over 350 cc. of a proprietary preparation of irradiated ergosterol in oil and over 270 cc. of a proprietary high potency vitamin A preparation in fifty-four days of life. Calcification of the kidneys, ascending meningitis and internal hydrocephalus were demonstrated at autopsy. The bones and the parathyroid glands of both infants were not decribed. The other 2 infants got both substances for eleven and twenty-two days but had no calcium deposits in their viscera.

Wolf [107] described a 3 month old boy who was born with spina bifida, lumbosacral meningomyelocele, paralysis of the lower extremities and an enlarged head. Between eight and 6 weeks before hospitalization, he had a temperature of 101 to 104 F., and the serum calcium was 11.5 mg. and the serum phosphorus 4.7 mg. per hundred cubic centimeters. During this time, he retained about 3,500,000 units of electrically activated ergosterol given in 300,000 unit daily doses, and he suffered from an infection of the scrotum due to coliform bacilli and streptococci. Over a period of six weeks, the scrotal infection subsided, the head increased in girth, and low temperatures and greatly elevated spinal fluid protein were observed. He died suddenly during a cisternal tap. Calcification of the kidneys, hydrocephalus, meningomyelocele, spina bifida and marasmus were found at necropsy. No mention was made of the bones or of the parathyroid glands.

Bauer and Freyberg [108] reported the case of a woman 32 years old, who suffered during the three years before her first hospitalization from recurrent attacks of diarrhea, malaise and headache. She was again admitted three years after discharge, complaining that there had been headaches, nasal obstruction and postnasal drip in the interim. For two years she had a hacking, mildly productive cough. For one year she took 100,000 units of vitamin D daily. From six to three months before admission she took 500,000 units or more daily. For three months she had swollen legs and feet, nodules on the extensor tendons of the fingers, pressure areas on the elbows and buttocks, thickened palms, pains in the extremities, easy fatigue, anorexia and loss of weight. Exam-

107. Wolf, I. J.: J. Pediat. **22**:707, 1943.

108. Bauer, J. M., and Freyberg, R. H.: J. A. M. A. **130**:1208, 1946.

ination disclosed: resting dyspnea; restlessness; pallor; distended cervical veins; diastolic gallop rhythm and transient rough diastolic murmur over the base of the heart; masses over the buttocks and sacroiliac joints; thickened, knotty palms; slight albuminuria; a hemoglobin content of 54 per cent; leukocytes 10,600 to 17,800 per cubic millimeter, with segmented neutrophils 82 per cent and lymphocytes 16 per cent; a sedimentation rate of 45 mm. per hour; serum albumin 3.7 Gm. and serum globulin 2.1 Gm. per hundred cubic centimeters; increased density of the lower lobe of the right lung by roentgenogram. Terminally there were cough, lethargy, epigastric pain, emesis, low grade fever and early gangrene of the right leg and foot. Autopsy revealed: calcification of the kidneys, the heart, the arteries, the lungs, the dura, the joints and the subcutaneous tissue; chronic duodenal ulcer; acute gastric and duodenal ulcers; chronic interstitial pancreatitis; perivascular cerebral hemorrhages; new-formed bone in the ribs and the sternum. The parathyroid glands were not described.

A man 44 years old, described by Mulligan,[109] had been taking large daily doses of a vitamin D preparation and alkaline salts for six months before he was admitted to the hospital. For two weeks he suffered from weakness, restlessness, drowsiness and alternate stupor and delirium. Examination showed: coma; a temperature of 99.8 F.; labored respiration; pallor; a loud blowing systolic precordial murmur; intact lungs; albuminuria; many granular casts and erythrocytes in the urine; a diffuse fine mottling of the lungs, by roentgenogram. Terminally there were irregular tachycardia, cough productive of abundant mucoid sputum, and continued coma. Autopsy disclosed: calcification of the kidneys, the heart, the arteries, the lungs, the stomach and the pancreas; pulmonary edema; hypertrophy of the heart and the kidneys; mural thrombosis of the left auricle; thrombosis of the renal veins; infarcts of the spleen; acute focal pancreatitis; chronic cholecystitis; cholelithiasis; slight fatty metamorphosis of the liver; osteoclasis of a rib; aspermiogenesis. The parathyroid glands were not examined. Chemical analysis revealed calcium phosphate in the kidneys and in the stomach and calcium carbonate in the lungs.

Summary.—Of the 9 patients in whom metastatic calcification was associated with hypervitaminosis D, 7 were infants and 2 were adults. Of the 7 whose sex was stated, 6 were males and 1 was a female. Vitamin D in some form played a chief causal role, and the kidneys were calcified in all 9 cases. The kidneys grossly were normal in size or enlarged, pale, swollen and yellow. The heart, the arteries and the lungs were calcified in 3 cases and the stomach in 2. The bones were not examined in 5 cases, were normal in 2, showed osteoclasis in 1 and new-

109. Mulligan, R. M.: Am. J. Path. **22**:1293, 1946.

formed bone in 1. A single parathyroid gland was normal in 1 case [108]; in all the others the parathyroid glands were not described. In 1 case chemical analysis demonstrated calcium carbonate in the lungs and calcium phosphate in the kidneys and the stomach.

METASTATIC CALCIFICATION AND UNCERTAIN ETIOLOGIC FACTORS

A man 29 years old, described by Grohe,[110] showed tuberculosis of the lungs, the meninges and the ileum, calcification of the colon, fibrosis of the liver, bronchitis, thinning of the calvarium and fibrous pleuritis at autopsy.

Chiari [8] observed severe pyloric stenosis and calcification of the lungs and the kidneys in a female of unstated age. The bones showed no anomaly.

Hlava [111] recorded the case of a woman 42 years old, who had a large inguinal hernia on the left side for twenty-four years, dyspnea and dry cough for one year, and insanity for one month before admission to a hospital. Terminally she showed continued vomiting, severe dyspnea and diffuse cutaneous emphysema. In addition to these findings, she had strangulation of loops of intestine in the hernial sac, emphysema and calcification of the lungs, rupture and atelectasis of the right lung, pneumothorax on the right, pneumoperitoneum and hypertrophy of the right side of the heart. The skull was not grossly remarkable. A chemical test indicated deposition of calcium carbonate in the lungs.

A woman 42 years old, described by Kischensky,[112] suffered during life from obesity, epigastric pain and severe vomiting. Calcification of the lungs and the stomach, edema of the lungs, fatty degeneration of the heart, amyloidosis of the spleen, brown atrophy of the liver, chronic interstitial nephritis and fibrous pleuritis were observed at autopsy. No skeletal disease was demonstrated. Chemical analysis revealed calcium acid phosphate in the lungs.

At autopsy a man 36 years old, described by Hedinger,[113] showed extensive calcification of viscera (heart, lungs, liver and kidneys), true contracted kidneys, widespread osteomalacia and cysts of the right humerus and the twelfth thoracic vertebra. The liver contained calcium phosphate by chemical test.

Liebscher [114] recorded the case of a 26 year old woman who at autopsy was found to have calcification of the heart, the arteries, the spleen and the liver and tuberculosis of the lungs and of the bronchial

110. Grohe, F.: Virchows Arch. f. path. Anat. **13**:277, 1858.

111. Hlava, J.: Wien. med. Bl. **36**:1099 and 1165, 1882.

112. Kischensky, D.: Centralbl. f. allg. Path. u. path. Anat. **12**:674, 1901.

113. Hedinger, E.: Cor.-Bl. f. schweiz. Aerzte **39**:833, 1909.

114. Liebscher, cited by Hedinger.[113]

and cervical lymph nodes. The hepatic deposits consisted largely of calcium phosphate. The bones were not mentioned.

The case reported by Surbek [115] was that of a girl born with a paretic right arm and dying of cardiac failure at the age of 2 days. Autopsy disclosed: calcification of the aorta, the systemic arteries, the lungs, the adrenal glands, the kidneys and the ovaries; chronic myocarditis, periarteritis, pancreatitis and myometritis; fatty metamorphosis of the liver; serofibrinous pericarditis. The humeri and the femurs were intact.

Harbitz [116] recorded the case of a woman 41 years old, who had two attacks of acute rheumatism. Later she had dyspnea, epigastric pain, hematemesis, epistaxis and menorrhagia for three years, cyanosis and swelling of the legs for several months, and orthopnea, enlarged abdomen and oliguria for seven weeks before she entered a hospital. Examination revealed: a pulse rate of 104 and a respiratory rate of 34 per minute; a blood pressure of 110 systolic; facial cyanosis; anasarca; clubbed fingers; cardiac enlargement; a systolic murmur loudest at the apex; an accentuated pulmonic second sound; prolonged respiratory expiration; hepatomegaly; ascites; blood erythrocytes 8,450,000 and leukocytes 11,600 per cubic millimeter. Terminally there was cardiac irregularity. Necropsy revealed: calcification and hypertrophy of the lungs; old tuberculosis of the lungs, the bronchial and cervical lymph nodes and the uterine tubes; hypertrophy of the right side of the heart; fibrous pleuritis; ascites. The bones and the parathyroid glands were not remarkable. Chemical analysis of the lungs showed 80 per cent calcium phosphate and 18 per cent calcium carbonate.

A boy described by Bross [117] died twenty minutes after birth and showed calcification of the lungs and a closed ductus arteriosus at autopsy. Chemical test of the lungs proved the presence of calcium salts. The bones were not remarkable.

A 6 day old girl was observed by Marsden [118] to have a generalized cutaneous rash of punched-out, crusted small ulcers, umbilical sepsis, a right scapular subcutaneous abscess, a temperature of 101 F., gradually progressive jaundice, manual and pedal spasm, paresis of the left side of the face, multiple hard subcutaneous nodules and cutaneous ecchymoses. In addition to these findings, autopsy revealed: calcification of the lungs, the liver, the adrenal glands, the kidneys, the thymus and the subcutaneous tissue; diffuse necrosis of the liver; tubular and glomerular nephritis; ulcers of the intestines; hemorrhages in the lungs, the adrenal glands and the kidneys; hemolytic streptococci in a left clavicular subcutaneous abscess and in the heart's blood; fibrous pleu-

115. Surbek, K.: Centralbl. f. allg. Path. u. path. Anat. **28**:25, 1917.
116. Harbitz, F.: Arch. Int. Med. **21**:139, 1918.
117. Bross, K.: Centralbl. f. allg. Path. u. path. Anat. **49**:229, 1930.
118. Marsden, J. P.: Brit. J. Child. Dis. **27**:193, 1930.

ritis, on the right side, and emaciation. Parathyroid gland tissue was abundant and active. A rib and a femur were normal.

The second patient of Grayzel and Lederer [27] was a married woman aged 25, who was delivered spontaneously at seven months of pregnancy, seven weeks before hospitalization. She had a dry cough for seven weeks, weakness, lassitude and vertigo for one month, and pyuria for two weeks. Examination showed: a temperature of 100.4 F.; a pulse rate of 120; a blood pressure of 124 systolic and 60 diastolic; a normal heart; decreased breath sounds over the lower lobe of the right lung; hepatomegaly; local tibial tenderness; hydrothorax on the right, by roentgenogram; albuminuria; a hemoglobin content of 53 per cent, erythrocytes 3,400,000 and leukocytes 6,700 to 24,400 per cubic millimeter, with stab neutrophils 4 to 20 per cent, segmented neutrophils 59 to 78 per cent and lymphocytes 12 to 28 per cent; serum calcium 15.6 mg., serum phosphorus 3.5 mg., serum phosphatase 8.2 units, serum albumin 2.3 Gm., serum globulin 2.4 Gm., blood urea 71 mg., blood uric acid 9.6 mg., blood cholesterol 154 mg., total lipids of blood 614 mg., plasma chlorides 316 mg. and serum sodium 280 mg. per hundred cubic centimeters; total base 140 milliequivalents; icterus index 3.6; carbon dioxide–combining power of blood 65.5 volumes per cent; a sedimentation rate of 210 mm. per hour; a myeloid-erythroid ratio of 80:20 in the bone marrow; nodules in the skin of the breasts, the axillas, the groins, the thighs and the right leg. Terminally there occurred fever (temperature of 105 F.), dyspnea, cyanosis, tachycardia, dependent edema, Cheyne-Stokes respiration and semistupor. Necropsy disclosed: calcification of the heart, the systemic arteries, the lungs, the liver, the skin and the larynx; infarcts of the spleen; chronic passive hyperemia of the liver; proctitis; cystitis; bilateral hydrothorax; a decubital ulcer over the right hip. The bones were not described. Three small parathyroid glands were microscopically normal. Chemical analysis of the cutaneous deposits showed 114 mg. of calcium and 1.6 mg. of phosphorus, compared with normal values of 0.1 mg. of calcium and 1.1 mg. of phosphorus.

An 8 week old girl, described by Baggenstoss and Keith,[119] was admitted to a hospital after she had suffered for several hours from vomiting, belching and distended abdomen. Examination showed severe dehydration, soft fontanels, a coated tongue and cold extremities. She died four days later. Cardiac hypertrophy, fatty metamorphosis of the liver and calcification of the heart, the systemic arteries and the kidneys were found at autopsy. Other organs were not remarkable.

The sixth case reported by Virchow in his first paper,[1] the second case detailed in his second paper,[4] the case of Babes [120] and Kaufman's

119. Baggenstoss, A. H., and Keith, H. M.: J. Pediat. **18**:95, 1941.
120. Babes, V.: Virchows Arch. f. path. Anat. **105**:511, 1886.

case[s] have been excluded from this collection of genuine examples of metastatic calcification. As a matter of fact, a few instances of this condition included in the clinicopathologic abstracts of cases accepted by me might be questioned by more severe critics.

Summary.—No systematic summary of the heterogeneous group of cases in this section has been attempted. Of the 12 patients included, 4 were infants and 8 were adults. Three were males and 9 were females. In 9 cases no hint of the etiologic basis for the calcification was given by the authors [121] who recorded them. Chronic interstitial nephritis may have been significant in causing calcification in the case reported by Kischensky,[112] but the evidence presented was too scanty for it to be classified specifically. The same was true for the case of Hedinger,[113] in which true contracted kidneys, widespread osteomalacia and cysts of a humerus and a vertebra were demonstrated to suggest that a parathyroid neoplasm may have been present and not investigated. Also to be considered is the possibility that the calcific deposits in the lungs of Harbitz' [116] patient represented dystrophic calcification on the basis of miliary tubercles. The parathyroid glands were not described in 8 cases [122] and were not remarkable in 4 cases.[123] Chemical analysis revealed calcium carbonate in the lungs,[124] calcium phosphate in the lungs [125] and the liver [126] and calcium salts in the lungs [117] and the skin.[27] Therapy with vitamin D or salts did not play a role in causing calcification in any of the 12 cases.

EXPERIMENTAL PRODUCTION OF METASTATIC CALCIFICATION

The reports of metastatic calcification produced by experimental use of extracts of parathyroid gland, vitamin D and minerals will be summarized, with preponderant emphasis on studies of the tissues of animals.

In dogs given injections of parathyroid extract, Hueper [127] observed psychic depression, vomiting, bradycardia, decreased blood coagulation time, hematemesis, melena, oliguria or anuria, slight albuminuria, weakness, dizziness, coma and death. At autopsy calcification involved occasional cardiac muscle fibers, a few elastic fibrils in the alveolar septums of the lungs, the gland cells of the fundic mucosa of the stomach, the circular muscle fibers of the duodenum, the basement membranes of

121. Grohe.[110] Chiari.[8] Hlava.[111] Liebscher.[114] Surbek.[115] Bross.[117] Marsden.[118] Baggenstoss and Keith.[119] Grayzel and Lederer.[27]

122. Grohe.[110] Chiari.[8] Hlava.[111] Kischensky.[112] Hedinger.[113] Liebscher.[114] Surbek.[115] Bross.[117]

123. Harbitz.[116] Marsden.[118] Baggenstoss and Keith.[119] Grayzel and Lederer.[27]

124. Hlava.[111] Harbitz.[116]

125. Kischensky.[112] Harbitz.[116]

126. Hedinger.[113] Liebscher.[114]

127. Hueper, W. C.: Arch. Path. **3**:14, 1927.

the glomerular capsules and tubules, the cells of the tubules, the casts in the lumens of the tubules of the kidneys and the colloid and the stroma of the thyroid gland. Also observed were acute gastric and duodenal ulcers, necrotic cells in the centers of the hepatic lobules and in the renal tubules, and hemorrhages in the stomach, the duodenum and the brain.

In 2 dogs receiving 100 to 150 units of a parathyroid extract, Learner [128] found serum calcium levels of 16.8 and 19.6 mg. per hundred cubic centimeters. Calcification affected the stroma and a few fibers of the heart, the intima of the coronary and splenic arteries, the elastic fibrils of the alveolar septums and the bronchi of the lungs, the basement membrane of the gland crypts and the parietal cells of the stomach, the cells and the sinusoids at the centers of the hepatic lobules, and the epithelial cells, the basement membranes and the lumens of the tubules, the walls of the arteries and the loops of glomeruli of the kidneys.

Large doses of a parathyroid extract injected subcutaneously into young growing guinea pigs by Jaffe, Bodansky and Blair [129] caused metastatic calcification in the heart, the lungs, the gastric and intestinal mucosa, the kidneys and the subcutaneous tissue. Generalized bone lesions, prominent at the costochondral junctions, the metaphyses and the diaphyses, included resorption of bone, degeneration and fibrosis of marrow and cessation of bone formation at the zones of active growth in forty-eight hours. Four days after the last injection, extensive subperiosteal callus and osteoid tissue were found.

Chown, Lee and Teal [130] injected parathyroid extract intermittently, both subcutaneously and intraperitoneally, into two strains of newborn rats. One strain was albino and afflicted with severe hydronephrosis in 0.5 per cent and mild hydronephrosis in 4 per cent of the animals. The other strain was hooded and had normal viscera. Within the first few days, granular calcium was found in the straight tubules, in the adjacent stroma or in the lumens of tubules. In rats given injections for fifteen to fifty-two days, the peritubular calcium masses were lacking. The albino rats showed greater calcium deposits. The same authors [131] reported on 147 rats given injections and 86 controls in longer experiments lasting up to one hundred and seventy-four days. Calcium did not involve the sclerotic glomeruli. In the first day the tubules and the pelves were dilated in relation to interstitial calcium deposits. In two days, tubular dilatation at the corticomedullary junction and peritubular fibrosis were noted. By thirteen days, some tubules were dilated and others collapsed within hyalinized basement membranes merging into the surrounding focally fibrotic, shrunken, chronically

128. Learner, A.: J. Lab. & Clin. Med. **14**:921, 1929.
129. Jaffe, H. L.; Bodansky, A., and Blair, J. E.: Arch. Path. **11**:207, 1931.
130. Chown, B.; Lee, M., and Teal, J.: Canad. M. A. J. **35**:513, 1936.
131. Chown, B.; Lee, M., and Teal, J.: Canad. M. A. J. **36**:7, 1937.

inflamed stroma. The calcium within the tubules increased with prolonged injection of the extract.

Cantarow, Stewart and Housel [132] injected 2,700 to 3,500 units of parathyroid extract intramuscularly into 5 dogs, 4 of which were female and 1 male. The serum calcium ranged between 17.5 and 20.3 mg. and the total serum proteins between 4.4 and 6.5 Gm. per hundred cubic centimeters. Calcification involved the heart, the arteries, the stomach and the kidneys. One female was pregnant with 9 fetuses 140 to 150 mm. long and showing no calcific or degenerative changes. The calcium deposits in the myocardium, the parietal cells of the gastric mucosa and the epithelial cells of the renal tubules were thought by the authors to be precipitated in degenerated tissue, although they did not explain why no calcification was found in degenerated areas of the thyroid gland, the liver and the skeletal muscles.

Kreitmar and Hintzelmann [133] gave 380 mg. of irradiated ergosterol to a cat in 19 days, during which a weight loss of 1.7 Kg. and terminal coma developed before the animal was put to death. Autopsy disclosed calcium deposits in muscle fibers and the stroma of the heart, in the intima and the media of the aorta, in all coats of the stomach, and in the basement membranes of glomeruli and convoluted tubules, the cells and the lumens of straight tubules, and the afferent glomerular arterioles of the kidneys.

Kreitmar and Moll,[134] by giving a proprietary preparation of irradiated ergosterol in oil to mice, rats, guinea pigs, rabbits, cats, and dogs, were able to produce calcification of the myocardium, the systemic arteries, the lungs, the stomach, the adrenal glands, the kidneys and the intercostal muscles as well as ulcers of the small intestine and severe atrophy of the spleen.

Brand and Holtz [135] gave 20 mg. of irradiated ergosterol in sesame oil daily to 96 rats divided into four groups, three of which were killed at five, ten and thirty-eight days. The average serum calcium value (11.8 to 16.9 mg. per hundred cubic centimeters) and phosphorus value (9.8 to 13.1 mg. per hundred cubic centimeters) rose with increasing doses, compared with a value of 9.8 mg. for calcium and a value of 8.3 mg. for phosphorus obtained on a control group of 24 rats divided in half and killed at ten and thirty-nine days. The fourth experimental group received 20 mg. of the drug daily for nine days, then only sesame oil for the next sixteen days, and were killed at thirty-eight days. The average serum calcium and phosphorus values for this group were

132. Cantarow, A.; Stewart, H. L., and Housel, E. L.: Endocrinology **22**:13, 1938.

133. Kreitmar, H., and Hintzelmann, U.: Arch. f. exper. Path. u. Pharmakol. **137**:203, 1928.

134. Kreitmar, H., and Moll, T.: München. med. Wchnschr. **75**:637, 1928.

135. Brand, T., and Holtz, F.: Ztschr. f. physiol. Chem. **185**:217, 1929.

12.4 mg. and 9.5 mg per hundred cubic centimeters. In the experimental animals calcification was found in the cardiac muscle fibers, in the media of the coronary arteries, in the elastic fibrils of the alveolar walls of the lungs, in the gland crypts and the stroma of the gastric mucosa, in the medulla of the adrenal glands and in the glomerular capsules and the basement membranes, epithelial cells and lumens of the tubules of the kidneys.

Two dogs given 5 to 7 million antirachitic units of a proprietary preparation of irradiated ergosterol in oil in eight weeks by Demole and Fromherz [136] showed a 20 per cent loss of body weight, and at autopsy calcium deposits were found in the endocardium of the left auricle and ventricle, in the intima of the pulmonic veins, in the intima and the media of the arch of the aorta, and in the glomeruli and the tubules of the kidneys.

Herzenberg [137] gave a proprietary preparation of irradiated ergosterol in oil in doses of 6 to 10 mg. to 12 adult rats for sixteen to forty-six days. The animals lost 20 to 25 per cent of their body weight, and constitutional symptoms developed. Necropsy revealed heavy calcium deposits in relation to necrotic areas in the heart muscle, the media of the aorta, the muscle of blood vessels, the muscle of the stomach and the diaphragm. Calcium deposits were noted in the elastic tissue of blood vessels, in the bronchial mucosa and the elastic tissue of the septums of the lungs, in the lamina propria of the trachea and in the glomerular capsules and basement membranes of the tubules of the kidneys. She was unable to decide whether necrosis of smooth muscle or injury with calcification of elastic fibrils was primary.

Smith and Elvove [138] gave 27 full-grown rabbits, orally or intramuscularly, irradiated ergosterol in doses of 1 to 10 mg. daily or total amounts of 29 to 310 mg. With doses over 2 mg. daily, there was a high mortality, and abundant calcium deposits were seen in the media of the thoracic aorta, in the bronchial cartilages and the interalveolar septums of the lungs and in the convoluted tubules and as casts in the straight tubules of the kidneys. Also seen was diffuse interstitial nephritis accompanying the calcium deposits. Chemical analysis of the lungs and the kidneys revealed an enormous increase of calcium, especially in the latter. The salt deposited was calcium phosphate. A high inorganic phosphorus level was produced by the larger doses of irradiated ergosterol. Elevated serum concentrations of phosphorus and calcium, even though the latter was not high in absolute amount, resulted in much tissue calcification. When the serum concentration of phosphorus was normal or low, abnormal deposits of calcium were not found in the

136. Demole, V., and Fromherz, K.: Arch. f. exper. Path. u. Pharmakol. **146**:347, 1929.

137. Herzenberg, H.: Beitr. z. path. Anat. u. z. allg. Path. **82**:27, 1929.

138. Smith, M. I., and Elvove, E.: Pub. Health Rep. **44**:1245, 1929.

tissues no matter how high the serum calcium was. The authors concluded that hypercalcemia alone was not enough to account for abnormal tissue deposits of calcium and that coincident high inorganic serum phosphorus was essential to such calcification.

Shohl, Goldblatt and Brown [139] fed 4 mg. of a proprietary preparation of irradiated ergosterol in oil daily for five to seven days to 10 rats, beginning when these were 7 weeks old. Autopsy showed: calcification in the myocardium, the cardiac blood vessels, the mucosa and muscle of the stomach and the tubules and blood vessels of the kidneys; parenchymatous degeneration in the liver and the kidneys; necrosis in the myocardium and in renal tubules, and infiltration-proliferation in the heart and the gastric smooth muscle. Two rats on a phosphorus-deficient diet and 2 on a calcium-deficient diet, as well as 5 control rats, showed no calcification. The authors thought that the degenerative changes preceded calcification in the heart, the stomach and the kidneys, although they conceded that true metastatic calcification was responsible for some of the deposition of calcium in these organs. They did not explain why calcium was not deposited in areas of the liver affected by parenchymatous degeneration.

Gough, Duguid and Davies [140] studied two groups of 18 full-grown young rats each, one on an acid diet, the other on an alkaline diet, both diets being equivalent in phosphorus content but low in calcium content. Twelve rats in each group also received daily 20,000 units of oral vitamin D as calciferol. Urinary calcium was relatively higher with the alkaline diet but was increased with both diets on the addition of calciferol. Renal calcium, determined on the left kidney, was highest in the rats on the alkaline diet-calciferol combination. The right kidney showed a gross white or yellow granular corticomedullary junction. Microscopically, calcification was most abundant in the animals getting calciferol and more severe with the alkaline diet. The chemical and histologic calcific changes were parallel. Nephrosis affected the rats on an acid diet and was enhanced by calciferol. The nephrotic changes included shrunken or necrotic epithelial cells, replacement fibrosis of atrophic tubules and dilatation of some remaining tubules and were thought by the authors to be independent of the calcific changes. Two other groups of 12 rats each were studied, one fed an acid diet, the other fed an alkaline diet. Six rats in each group also received calciferol. The average excretion of urinary phosphorus was relatively elevated with the acid diet, lowered with the alkaline diet and much lowered with both diets on the addition of calciferol. The acid diet-calciferol combination resulted in the largest content of renal phosphorus.

139. Shohl, A. T.; Goldblatt, H., and Brown, H. B.: J. Clin. Investigation **8**:505, 1930.

140. Gough, J.; Duguid, J. D., and Davies, D. R.: Brit. J. Exper. Path. **14**:137, 1933.

Tanaka [141] injected a calcium lactate solution intraperitoneally into a rabbit and observed deposition of calcium in the peritoneum, in the muscle fibers of the heart, in the intima and the media of the aorta, in the lumens of the straight tubules of the kidneys and in the skeletal muscles of the extremities. He also gave 4 dogs intravenously calcium lactate and sodium phosphate seven to eighteen days after removing 200 cc. of blood by vein from each. Within twenty-four hours after the injection, autopsy revealed calcification of epithelial cells in the renal tubules in 4, of muscle fibers in the heart in 4, of the capsule of the spleen or the liver in 2 each, of the endocardium in 1 and of the stomach in 1. Hemorrhages involved mucous and serous surfaces in all 4.

Rabl [142] fed 17 mice for three to five days on an alternating acid and alkaline diet containing one part of tertiary calcium phosphate. Calcification involved: heart muscle fibers; all coats of the aorta and large arteries; elastic fibrils, bronchi, bronchial cartilages and veins in the lungs; the tunica propria, the gland cells and lumens of glands in the stomach; the basement membranes and the lumens of the renal tubules.

Butler [39] gave 9 mice an acid diet for five to sixteen days and found calcium deposits in the lungs, the stomach and the kidneys varying from none or a trace to grades III or IV. Six other mice fed an alkaline diet for eight to fourteen days showed calcification of the same organs varying from none or a trace to grades II or III. Eleven mice fed an alternating acid and alkaline diet for four to sixteen days had calcium deposits in these organs varying from none or a trace to grades III or IV. Microscopic study revealed calcification of the alveolar septums, the intima of veins and arteries and the basement membranes of the bronchioles in the lungs, of the mucosal glands in the stomach and of epithelial cells and basement membranes in the renal tubules. In 5 animals deposition of calcium was found in the myocardium. In 17 control mice no calcification was observed except in a small area in the renal collecting tubules. No inflammatory reaction accompanied the calcium deposits in the experimental animals.

Dreyfuss [143] gave tertiary calcium phosphate to four groups of mice. Of 6 mice fed an alternating acid and alkaline diet for six to fifty-four days, calcification affected the ventricular cardiac muscle fibers in 5, the bronchial cartilages in 1, the gastric mucosa in 6 and lumens of the renal tubules in 6. Of 7 mice fed an acid diet for two to forty-five days, calcium deposits involved the cardiac muscle in 6, the intima and the media of the blood vessels and the bronchial cartilages of the lungs in 7, the gastric mucosa in 6 and the lumens of the renal tubules in 7. The cardiac and renal deposits were much heavier than in the group fed the alternating diet. Of 6 mice fed an alkaline diet for eleven to thirty-eight

141. Tanaka, M.: Biochem. Ztschr. **35**:113, 1911.
142. Rabl, C. R. H.: Virchows Arch. f. path. Anat. **245**:542, 1923.
143. Dreyfuss, W.: Beitr. z. path. Anat. u. z. allg. Path. **76**:254, 1926-1927.

days, calcium deposits marked the cardiac muscle fibers in 2, the intima of blood vessels and the bronchial cartilages of the lungs in 3, the muscularis of the stomach in 1 and the kidneys in 5. The heart, the lungs and the stomach were much less affected than in the groups on the alternating or the acid diets and the kidneys were less involved than in the group on the alternating diet. Of 5 mice fed a neutral diet for an unstated period, calcification involved a few cardiac muscle fibers in 1, a large vein and a bronchial cartilage of the lungs in 1 and the kidneys in 5. The stomach was not calcified in any of the 5 mice in this group, in which renal calcification was about as intense as that in the animals fed the alkaline diet.

Kleinmann [144] fed 57 adult mice various diets. The most convincing results were observed in three groups. Of 4 mice fed an alternating acid and alkaline diet for eleven to nineteen days, calcium deposits were noted in cardiac muscle fibers in 1, in the walls of large cardiac blood vessels in 3, in the elastic fibrils of the alveolar walls of the lungs in 4, in the gastric mucosa in 2, and in the lumens of the renal tubules in 2. Of 4 mice fed an acid diet for sixteen to thirty-two days, calcification involved cardiac muscle fibers in 4, the alveolar walls of the lungs in 4, the gastric mucosa in 1, and the lumens and basement membranes of the tubules and the stroma of the kidneys in 4. Of 2 mice fed an alternating acid and alkaline diet (supplemented with calcium chloride instead of with calcium phosphate as in the other two groups) for five and twelve days, calcium deposits affected cardiac muscle fibers and stroma in 2, the alveolar walls of the lungs in 2, the gastric mucosa in 1 and the epithelial cells and the lumens of the tubules and the stroma of the kidneys in 2.

Stephens and Barr [145] fed adult rats various diets and put all animals to death after fifteen days. Of 4 rats on an acid, high calcium, high phosphorus diet, the heart was calcified in 2, the pulmonary, renal and gastric arteries in 3, the stomach in 2 and the kidneys in 4. Of 4 rats fed an alternating acid and alkaline, high calcium, high phosphorus diet, the pulmonary arteries were calcified in 3 and the kidneys in 4. The heart and stomach were not affected by calcification. Of 5 rats fed an alkaline, high calcium, high phorphorus diet, 5 fed a neutral, high calcium, high phosphorus diet, 12 fed an acid, high calcium diet, 6 fed an alternating acid and alkaline, high calcium diet, 6 fed an acid, high phosphorus diet and 6 control rats calcification was absent from the viscera in all. Microscopically, the calcific deposits were chiefly extracellular, were usually encircled by lymphocytes and consisted mainly of tertiary calcium phosphate as shown by chemical tests. The deposits were found in the myocardium, in the intima and the media of the pulmonary, renal and gastric arteries, in the muscle coat of the stomach and

144. Kleinmann, H.: Virchows Arch. f. path. Anat. **268**:686, 1928.

145. Stephens, D. J., and Barr, D. P.: Proc. Soc. Exper. Biol. & Med. **30**:920, 1933.

in the tubules and the interstitial tissue of the kidneys. Calcium was found in bronchial cartilages in both experimental and control animals. The walls of the alveoli of the lungs were not specifically described as affected by calcification.

Summary.—Metastatic calcification has been produced in dogs,[146] guinea pigs,[129] and rats [147] by injections of parathyroid extract. The organs involved by deposition of calcium were usually those affected in human beings with primary parathyroid neoplasm, although chronic experiments comparable to the spontaneous disease in man were not observed. Degenerated liver cells were found,[146] but calcification was observed in only one experiment.[128] The degenerative, inflammatory and fibrotic changes accompanying the calcification in the kidneys were emphasized.[131] Elevated serum calcium levels were noted,[148] but attention to inorganic serum phosphorus was lacking.

Large doses of vitamin D have caused metastatic calcification in rats,[149] dogs,[150] cats,[151] rabbits,[152] guinea pigs [134] and mice.[134] The deposits were ordinarily distributed as are those observed in human beings affected by metastatic calcification due to vitamin D or other causes, and resembled those closely. Calcium deposits were associated with degenerative changes in two experiments.[153] Degenerative and fibrotic changes were severe in the kidneys of rats given an acid diet and vitamin D.[140] Diffuse interstitial nephritis accompanied the renal calcification.[138] Calcium phosphate [138] was the salt deposited in the lungs and the kidneys, the latter organs containing more of the compound. The calcium and the inorganic phosphorus of the serum were increased in 2 instances,[154] and were increased further as more vitamin D was given. The amounts of urinary and renal calcium paralleled each other and were highest when an alkaline diet was given with vitamin D.[140] The urinary phosphorus was lowered to the greatest degree by an alkaline diet plus vitamin D, and the renal level of phosphorus with the same combination was lower than the maximal level of renal phosphorus reached when an acid diet and vitamin D were employed.

Minerals have been used to produce metastatic calcification in the mouse,[155] the rabbit,[141] the dog [141] and the rat.[145] In distribution and character the calcific deposits were similar to those seen in animals

146. Hueper.[127] Learner.[128] Cantarow.[132]

147. Chown, Lee and Teal (footnotes 130 and 131).

148. Learner.[128] Cantarow and others.[132]

149. Kreitmar and Moll.[134] Brand and Holtz.[135] Herzenberg.[137] Shohl and others.[139] Gough and others.[140]

150. Kreitmar and Moll.[134] Demole and Fromherz.[136]

151. Kreitmar and Hintzelmann.[133] Kreitmar and Moll.[134]

152. Kreitmar and Moll.[134] Smith and Elvove.[138]

153. Herzenberg.[137] Shohl and others.[139]

154. Brand and Holtz.[135] Smith and Elvove.[138]

155. Butler.[89] Rabl.[142] Dreyfuss.[143] Kleinmann.[144]

receiving parathyroid extract or vitamin D, except that degenerative tissue changes were not observed. Tertiary calcium phosphate was demonstrated in the calcified tissues by chemical test.[145] Except for one experiment of a rather acute nature,[141] the work with minerals has been based on a dietary approach.[156] An acid diet, closely followed by an alternating acid and alkaline diet, has been more effective in causing calcification of tissues than an alkaline or a neutral diet. Adequate amounts of calcium and phosphorus, especially of the latter,[145] have been necessary in the diets to produce the calcific changes.

MECHANISMS CONCERNED IN METASTATIC CALCIFICATION

In regard to bone disease as a cause of metastatic calcification, still valid is the original concept of Virchow [1] that the calcium salts derived from the breakdown of osseous tissue enter the blood stream in high concentration, principally as phosphates and carbonates, and are then precipitated in those tissues most susceptible to calcification. Several factors are involved in this process. The first is the composition of bone. According to Taylor and Sheard,[157] the solid phase of bone is composed of minerals of the apatite series of the formula $3Ca_3(PO_4)_2 \cdot CaX_2$, in which X_2 ordinarily represents CO_3, F_2, $(OH)_2$, O, or SO_4 and the Ca may be to some extent replaced by Mg. They found that the residual Ca : P ratio of bone is 1.94 and that the roentgenographic patterns and optical properties of bones and naturally occurring minerals of similar composition, including podolite, dahllite and fluorapatite, are the same. In normal rat bone Kramer and Shear [158] found a residual Ca : P ratio of 1.99. They observed that the proportion of carbonate calcium to total calcium was 8 to 10 per cent in the bones of young rats, compared with 15 to 16 per cent in those of adult rats. In primary calcification of the older bones of both young and adult animals, the proportion of carbonate calcium was less and a residual Ca : P ratio of 2.23 was observed, indicating to them [158] that a basic calcium salt is present when bone salts are freshly deposited. Normal adult bone has been found to consist [159] of about 80 per cent calcium phosphate, 13 per cent calcium carbonate, 2 per cent magnesium phosphate and a residue of calcium or magnesium fluoride, oxide, hydroxide or sulfate and magnesium carbonate.

The second factor is the manner in which calcium salts are released from bone. In the destruction of bone by osseous lesions, not only is the organic matrix of the bone directly destroyed to release calcium salts, but hyperemia is one component of the accompanying inflammatory reaction. Blair [160] presented the theory that alternating ischemia and hyper-

156. Rabl.[142] Dreyfuss.[143] Kleinmann.[144] Stephens and Barr.[145]
157. Taylor, N. W., and Sheard, C.: J. Biol. Chem. **81**:479, 1929.
158. Kramer, B., and Shear, M. J.: J. Biol. Chem. **79**:147, 1928.
159. Schmidt, C. L. A., and Greenberg, D. M.: Physiol. Rev. **15**:297, 1935.
160. Blair, H. C.: Surg., Gynec. & Obst. **67**:413, 1938.

emia maintain normal calcification of bone, aid in the healing of fractures and are produced by contraction and relaxation of skeletal muscles. Grieg [161] stated that if the circulation is maintained within normal limits, bone remains unchanged, but that definite hyperemia causes bone to undergo rarefaction, decalcification and osteoporosis, contrasted with ischemia, which results in calcification and ossification of bone. As bone is destroyed, the organic matrix may furnish protein molecules with which calcium ions may combine, although increased concentration of hydrogen ions could inhibit this process to some extent by driving it in the direction of ionization of calcium proteinate so formed. With the increasing acidity of the inflammatory process accompanying destruction of bone, the high local concentration of carbon dioxide could enhance the solution [159] of calcium salts. Kay [162] demonstrated a rise in the plasma phosphatase in cases of osteomyelitis, osteitis deformans, metastatic carcinoma of bone, sarcoma of bone, osteitis fibrosa cystica and other conditions. He expressed the opinion that the increase of plasma phosphatase is probably due to the bone disease, possibly because it is produced in excessive amounts in an attempt to compensate for the bony lesion. Kay [163] concluded that the phosphatase derived from bone is able to hydrolyze many monophosphoric esters and that such esters, which form soluble calcium salts, may serve as a substrate for the precipitation of calcium phosphate in ossification. On the other hand, phosphatase may be able to synthesize [163] phosphoric esters from organic compounds, e. g., glycerol, under conditions in which even low concentrations of inorganic phosphate are found in the tissues, thus reversing the equation

$$\text{Phosphoric ester} \underset{\longleftarrow}{\xrightarrow{\text{phosphatase}}} \text{inorganic phosphate} + \text{organic compound}$$

By this mechanism, calcium salts could be transferred from bones with directly destructive lesions according to the following equations, which employ glycerol as a sample organic compound, the sodium phosphate buffers of the blood, and the calcium orthophosphate ($Ca_3[PO_4]_2$) and calcium carbonate ($CaCO_3$) released by the breakdown of the organic matrix of the bone:

(1) $Ca_3(PO_4)_2 + 4NaH_2PO_4 + C_3H_8O_3 \xrightarrow{\text{phosphatase}} 3CaHPO_4 + 2Na_2HPO_4 + C_3H_7O_3(H_2PO_4) + H_2O$

or

$$3Ca^{++} + 2PO_4^{\equiv} + 4Na^{+} + 4H_2PO_4^{-} + CH_2OH{\cdot}CHOH{\cdot}CH_2OH \xrightarrow{\text{phosphatase}} 3Ca^{++} + 5HPO_4^{\equiv} + 4Na^{+} + CH_2OH{\cdot}CHOH{\cdot}CH_2(H_2PO_4) + H_2O$$

(2) $CaCO_3 + NaH_2PO_4 \longrightarrow CaHPO_4 + NaHCO_3$

or

$$Ca^{++} + CO_3^{\equiv} + Na^{+} + H_2PO_4^{-} \longrightarrow Ca^{++} + HPO^{\equiv} + Na^{+} + HCO_3^{-}$$

161. Grieg, D. M., cited by Blair.[160]
162. Kay, H. D.: J. Biol. Chem. **89**:249, 1930.
163. Kay, H. D.: Physiol. Rev. **12**:384, 1932.

The release of magnesium ions by the breakdown of the osseous tissue would catalyze [164] the action of the phosphatase.

The third factor involved is the blood transport of calcium, phosphorus and magnesium whereby these elements reach the tissues susceptible of being calcified in the metastatic process. Tertiary calcium phosphate and calcium carbonate from the bones may be transformed as indicated into monohydric calcium orthophosphate ($CaHPO_4$), which is transported in the blood as suggested by the experiments of Shear and Kramer.[165] On mixing solutions containing calcium with solutions containing phosphate so that the resulting solution was acid, they observed the formation of a precipitate, $CaHPO_4$. Inorganic serum solutions were adjusted to a p_H of 7.3 with calcium to the concentration of 10 mg. per hundred cubic centimeters and phosphorus to concentrations varying from 1.5 to 6.0 mg. per hundred cubic centimeters. When the Ca $\times$ P product was 40, calcification occurred, but when this figure dropped to 30, no calcification was obtained. The critical value was 35. When the solubility product constant ($K_{s.p.}$) of $(Ca^{++}) \cdot (HPO_4^{=})$ was between 2.7×10^{-6} and 4.0×10^{-6}, the empiric product of Ca $\times$ P was 40 to 60. This constant was lowered in proportion to the drop of the Ca $\times$ P product. When the p_H of the serum differed to any degree from 7.3, the $CaHPO_4$ was not held in solution. When the p_H dropped to 7 or slightly below, the solubility product constant was so low that the ion products of $CaHPO_4$ were undersaturated and calcification was not obtained even with a Ca $\times$ P product as high as 50. They concluded that normal serum is probably very near to saturation with $CaHPO_4$. By shaking crystalline $CaHPO_4$ with inorganic serum solutions, Shear, Washburn and Kramer [166] found the $(Ca^{++}) \cdot (HPO_4^{=})$ ion product at equilibrium, even though the solid phase was in excess and was independent of the initial concentration of Ca and P. The mean value for the solubility product constant of $CaHPO_4$ at 38 C. was $3.4 \times 10.^{-6}$ They also observed that inorganic serum solutions with an empiric Ca $\times$ P product of less than 50 were undersaturated with respect to $CaHPO_4$, while those with a value above 50 were supersaturated, making 50 the critical level at which calcification occurred. In addition to $CaHPO_4$, serum contains calcium proteinate and ionic calcium; the calcium combined with protein is combined mainly with the albumin fraction of the serum.[159] Phosphorus is present in serum as inorganic phosphate, nucleotides, glycerophosphate, phosphoric esters, glycerophosphoric acid and phospholipids.[159] Magnesium exists in serum in the same three states as calcium.[159]

164. Jenner, H. D., and Kay, H. D.: J. Biol. Chem. **93**:733, 1931.

165. Shear, M. J., and Kramer, B.: J. Biol. Chem. **79**:125, 1928.

166. Shear, M. J.; Washburn, M., and Kramer, B.: J. Biol. Chem. **83**:697, 1929.

The fourth factor involved is the mechanism by which calcium salts are precipitated in those tissues especially prone to be alkaline because of the low carbon dioxide content or, conversely, because of the high oxygen content of the blood bathing them—such as the capillaries of the alveoli, the venules and the veins of the lungs, the left chambers of the heart, and the systemic arterial tree. In those organs in which acids are excreted, such as the lungs (CO_2), the gastric mucosa (HCl) and the kidneys (acid PO_4), the tissues are rendered alkaline and similar precipitation of calcium salts occurs.[3] Since serum is normally saturated [166] with respect to $CaHPO_4$ and an alkaline p_H favors [165] the holding of this compound in solution, the following reactions could express the means by which $CaHPO_4$ is changed to $Ca_3(PO_4)_2$ and $CaCO_3$ for deposition in the soft tissues:

(3) $3CaHPO_4 + Na_2HPO_4 \longrightarrow Ca_3(PO_4)_2 + 2NaH_2PO_4$

or

$3Ca^{++} + 4HPO_4^{=} + 2Na^{+} \longrightarrow 3Ca^{++} + 2PO_4^{\equiv} + 2H_2PO_4^{-} + 2Na^{+}$

(4) $CaHPO_4 + NaHCO_3 \longrightarrow CaCO_3 + NaH_2PO_4$

or

$Ca^{++} + HPO_4^{=} + Na^{+} + HCO_3^{-} \longrightarrow Ca^{++} + CO_3^{=} + Na^{+} + H_2PO_4^{-}$

The monosodium acid phosphate (NaH_2PO_4) could be returned to the bone and would facilitate the reactions in equations (1) and (2) for the transformation of $Ca_3(PO_4)_2$ and $CaCO_3$ of the bone to $CaHPO_4$ for blood transport. The glycerophosphates or other phosphoric esters in the serum and in the tissues becoming calcified could be an available substrate for the action of phosphatase elevated in the serum [162] toward hydrolysis [167] of these esters and the production of phosphate ions to enhance calcification. However, no evidence has been found to prove that phosphatase acts in tissues undergoing calcification by the metastatic process, even though the low concentration of hydrogen ions in these tissues would favor its action at an optimal p_H of 9 as compared with a p_H of 5, which inactivates this enzyme.[163] The renal cortex has a high degree of phosphatase activity,[168] which might be concerned with the deposition of calcium salts in the kidneys by the same mechanism. In experiments on frog muscle and canine carotid arteries, which were injured, Burge and co-workers [169] demonstrated that the injured portion was rendered electronegative to the uninjured portion. Increased phosphate was observed in the injured part of the frog muscle by the use of ammonium molybdate. When calcium chloride or barium chloride in twice-normal solution was applied to the injured parts, precipitation of

167. Robison, R.: Biochem. J. **17**:286, 1923.

168. Kay, H. D.: Biochem. J. **20**:791, 1926.

169. Burge, W. E.; Orth, O. S.; Neild, H. W.; Ash, J., and Krouse, R.: Arch. Path. **20**:690, 1935.

calcium phosphate or of barium phosphate resulted, and the current of a few microamperes was abolished. They concluded that calcification of arteries may result from a combination of positively charged calcium ions of the blood with negatively charged phosphate ions in the tissues, so that calcium phosphate is precipitated. This mechanism could be operative in metastatic calcification of tissues, particularly after direct calcification had already involved them, thus allowing phosphate ions to be released toward further deposition of calcium phosphate.

The fifth factor involved concerns the types of salts deposited in the soft tissues and the ratio of Ca : P in them. Kramer and Shear [170] examined 7 specimens of tissues pathologically calcified by the dystrophic process and found a residual Ca : P ratio of 1.86 to 2.01. In three calcified fibroids, values of 2.18 to 2.23 were noted. Similar quantitative studies on tissues pathologically calcified by the metastatic process have not been found, but the supposition that this ratio would be the same is allowable. Among the patients with bone disease and metastatic calcification, calcium carbonate was found in the lungs of 5, the hearts of 3, the kidneys of 2, the gastric mucosa of 1 and the skin of 1. Calcium phosphate was noted in the lungs of 3, the gastric mucosa of 1, the heart of 1 and the kidneys of 1.

Impaired in chronic renal disease, because of the destruction of renal parenchyma, is the ability of the body to excrete inorganic phosphorus, as well as the phosphate of phosphoric esters through renal phosphatase activity, by way of the kidneys, the main avenue whereby phosphorus compounds are excreted [159] as acid phosphates. Thus phosphate is built up in the blood to levels higher than normal, even to extreme levels.[61] With this rise in phosphate, a reciprocal drop in blood calcium occurs to maintain the $(Ca^{++}) \cdot (HPO_4^{=})$ solubility product constant and the empiric Ca × P product. In an effort to enhance the excretion of phosphate in the greatly reduced surviving renal parenchyma, the parathyroid glands become enlarged as observed in 15 of the 23 patients with chronic renal disease and metastatic calcification. Eleven of them showed diffuse chief cell hyperplasia of the parathyroid glands, characteristic of chronic renal disease.[49] That this same abnormality of the parathyroid glands may result from inadequate absorption of calcium and phosphate from the intestine has been little appreciated.[171] Through the overactivity of the hyperplastic parathyroid glands in chronic renal disease, the blood calcium is increased [172] so that the serum becomes supersaturated with both calcium and phosphate and precipitation of calcium salts occurs in the soft tissues. The level of phosphate is more important, for although

170. Kramer, B., and Shear, M. J.: J. Biol. Chem. **79**:121, 1928.

171. Mulligan, R. M.: Arch. Path. **40**:182, 1945.

172. Hubbard and Wentworth.[38] Smyth and Goldman.[44] Price and Davie.[48] Brown and Ginsberg.[51] Herbert and others.[52]

normal,[59] normal or depressed [53] or even decreased [43] serum calcium values may be observed, metastatic calcification occurs if the phosphate in the serum is elevated. The calcium of the blood and the accompanying phosphate and carbonate ions have their source in the bones, in which osteitis fibrosa cystica was the lesion in 7 patients, rickets in 4 and osteoporosis in 2 patients of the 13 with chronic renal disease whose bones were examined histologically. The direct action of parathyroid hormone; the hyperemia attendant on passive visceral hyperemia caused by a hypertrophied failing heart, often seen in chronic renal disease; the high local carbon dioxide content resulting from decreased carbon dioxide–combining power of the blood [66]; the local increase of protein due to the breakdown of organic bony matrix; the elevated phosphatase [62] all may play etiologic roles when calcium salts are being mobilized from the bones in chronic renal disease. Equations (1) and (2) could explain the removal of these salts as in the case of bone disease. With calcium and phosphate as well as carbonate being increased in the blood as they are wihtdrawn from the bones, the elevation of tissue p_H, the saturation of the serum with monohydric calcium orthophosphate ($CaHPO_4$), the action of alkaline phosphatase on phosphoric esters, equations 3 and 4 as given in a foregoing paragrgaph, and initial injury of tissue by direct calcification would have to be considered in explaining tissue calcification in the case of chronic renal disease as they are in the case of bone disease. Of the 23 patients with chronic renal disease whose cases were reviewed, 43 per cent had calcified kidneys. Although elevated concentration of hydrogen ions of the blood is a frequent feature of chronic renal disease, episodes of vomiting, also observed, may result in enough chloride being lost from the blood to cause temporary elevation of the blood bases, so that tissue alkalinity, especially in those sites prone to be alkaline, is intermittently raised. This would tend to have an effect like that of alternating acid and alkaline diets [173] in causing calcium to be deposited in the tissues. In the heart Schmidt [37] found calcium phosphate and a preponderance of calcium in the left ventricle as compared with the right ventricle. Smyth and Goldman [44] observed periarticular deposits of calcium phosphate and a ratio of calcium to phosphorus of about 2:1 in the long bones of their patient. Pons and Pappenheimer [50] noted that the amount of calcium in the kidneys of their second patient was about one hundred and thirty times the amount found in normal kidneys.

In primary hyperparathyroidism [174] resulting from a chief cell or other type of adenoma of a parathyroid gland or from diffuse hyperplasia of wasserhelle cells of two or more parathyroid glands, both calcium and phosphate are withdrawn from the bones,[159] conspicuously from the

173. Butler.[39] Rabl.[142] Dreyfuss.[143] Kleinmann.[144] Stephens and Barr.[145]
174. Castleman, B., and Mallory, T. B.: Am. J. Path. **11**:1, 1935.

trabeculae,[175] and are discharged into the blood by the great activity of the neoplastic parathyroid tissue. The mechanisms operative in the removal of calcum salts in bone disease and chronic renal disease could be active in primary hyperparathyroidism, such as the hyperemia associated with new-formed osteoid tissue,[78] increase of alkaline phosphatase [162] and a high local concentration of protein following the breakdown of original organic bony matrix. Equations (1) and (2) could also apply. By the action of parathyroid hormone the blood calcium is raised [176] and the blood inorganic phosphorus lowered,[176a] although both are excreted by the kidneys in increased amounts,[176] especially phosphorus. Fecal excretion of calcium is not affected.[176a] Muscle weakness [87] is caused when the calcium ions of the blood are increased; renal calculi,[174] when calcium phosphate is being excreted in large amounts by the kidneys; osteitis fibrosa cystica,[174] when bone is being resorbed and replaced by fibrous and osteoid tissue,[73] and metastatic calcification, when calcium salts are being deposited in the soft tissues. In patients with primary hyperparathyroidism [174] the formation of renal calculi precedes osteitis fibrosa cystica by a long enough interval to indicate that the calcium salts are withdrawn from the bones at an early stage of the disease and that the osseous lesion is manifest only in the later stages. The lesion in the bones of 17 of the 21 patients with primary neoplasm of the parathyroid glands reviewed was osteitis fibrosa cystica in 14, osteomalacia in 1, osteoporosis in 1 and osteosclerosis in 1. A superb presentation of the genesis of osteitis fibrosa cystica has been given by Dawson and Struthers.[73] The experimental production of this lesion has been observed in guinea pigs by Jaffe, Bodansky and Blair [129] and in albino rats by Selye.[177] The kidneys of 90 per cent of the 21 patients with primary parathyroid neoplasm and metastatic calcification were calcified to various degrees, and more or less inflammatory reaction, degenerative changes and fibrosis accompanied the deposition of calcium salts. Apparently the kidneys are the first site of calcification in primary hyperparathyroidism, and when the destructive alterations accompanying this calcification have supervened, the amount of normal renal parenchyma is greatly reduced and the excretion of phosphate especially is impaired as in primary chronic renal disease. When this occurs, or even when the blood calcium is raised [176a] above a critical level of 14 to 15 mg. per hundred cubic centimeters of serum, the excretion of urinary phosphate falls and the level of blood phosphate is abnormally high. With the blood calcium already increased, the critical level of both ions is raised in the blood to the point at which precipitation occurs in those

175. Bauer, W.; Aub, J. C., and Albright, F.: J. Exper. Med. **49**:145, 1929.

176. (*a*) Albright, F.; Bauer, W.; Ropes, M., and Aub, J. C.: J. Clin. Investigation **7**:139, 1929. (*b*) Schmidt and Greenberg.[159]

177. Selye, H.: Endocrinology **16**:547, 1932.

tissues, besides the kidneys, prone to direct calcification. With the reduction of renal parenchyma by calcific and secondary pathologic changes, the already neoplastic parathyroid tissue is incited to further efforts toward facilitating excretion of phosphate and calcium is further built up in the blood by the action of parathyroid hormone. Thus a vicious circle is set up by renal damage superimposed on the original overactivity of the cells of an adenoma or of hyperplastic water-clear cells. Calcification of the soft tissues in primary hyperparathyroidism depends on the hydrogen ion concentration of the tissues calcified, the saturation of serum with monohydric calcium orthophosphate ($CaHPO_4$), the local action of phosphatase elevated in the serum (and in the surviving renal parenchyma) on phosphoric esters, and probably to some degree on actual primary injury of tissue as described by some authors.[178] Equations 3 and 4 may also be operative. The renal calculi found in primary hyperparathyroidism are usually calcium phosphate.[174] Chemical data on tissue deposits of calcium salts have been absolutely lacking.

Excessive doses of vitamin D have produced elevation of both calcium [179] and inorganic phosphorus [154] in the serum by causing both to be withdrawn from the bones.[150] In dogs given large doses of irradiated ergosterol, Taylor and Weld [179a] observed a fall in fecal calcium, a rise in serum calcium, an increased urinary excretion of calcium and a negative calcium balance even when a low calcium diet was fed. The net result of excessive doses of vitamin D is retention of calcium [180] and phosphorus,[181] so that the solubility product constant of $(Ca^{++}) \cdot (HPO_4^{=})$ is relatively easily exceeded and precipitation of both occurs in those soft tissues susceptible to metastatic calcification. Calcium salts are removed from the bones by vitamin D mainly through osteoclasis.[182] Jones and Robson [182b] observed that in rats 68 to 138 days old which had been given irradiated ergosterol for fifty-two to sixty-three days, the femur, the tibia and the fibula showed extensive osteoclastic resorption, especially of the cortical bone of the diaphyses. The process did not seem to be simple halisteresis but destruction and removal of the entire bone matrix brought about by the action of osteoclasts. The process began along the inner margin of the cortex of the shaft and extended outward. Where bone had been destroyed, the

178. Oliver.[84] Hueper.[127] Cantarow and others.[132]

179. (*a*) Taylor, N. B., and Weld, C. B.: Brit. J. Exper. Path. **13**:109, 1932. (*b*) Brand and Holtz.[135] (*c*) Smith and Elvove.[138]

180. Brand and Holtz.[135] Smith and Elvove.[138] Gough and others.[140] Schmidt and Greenberg.[150] Taylor and Weld.[179a]

181. Brand and Holtz.[135] Smith and Elvove.[138] Gough and others.[140] Schmidt and Greenberg.[150]

182. (*a*) Grauer, R. C.: Proc. Soc. Exper. Biol. & Med. **29**:466, 1932. (*b*) Jones, J. H., and Robson, G. M.: Am. J. Physiol. **103**:338, 1933. (*c*) Mulligan.[109] (*d*) Schmidt and Greenberg.[150]

spaces left were filled up by marrow. Hyperemia caused by this extension of marrow and high local concentration of protein following the destruction of organic bone matrix could help to remove calcium salts from the bones in hypervitaminosis D. Data, especially with reference to human beings, on alkaline phosphatase in this condition are not available to indicate either a positive or a negative role for the enzyme in osseous breakdown. Baumgartner, King and Page [183] showed bone phosphatase greatly decreased in rabbits 9 to 12 months old which were given a proprietary preparation of irradiated ergosterol in oil for two to five and one half months. Rabbits given excessive amounts of irradiated ergosterol showed no phosphatase activity in arteries which were the sites of calcification.[163] On the other hand, Page [184] demonstrated decreased phosphatase activity in the bones of rats given injections of a parathyroid extract as contrasted with the elevated serum phosphatase observed by Kay [162] in patients with osteitis fibrosa cystica. Although it has been concluded [185] that the actions of parathyroid hormone and vitamin D are parallel in many ways, this similarity does not imply identity. The differences in the bony lesions, in the changes in the blood calcium and phosphorus and in the effects on the fecal calcium produced by these substances indicate definite variations in their behavior, to name a few. In this laboratory, atrophy [186] of the parathyroid glands has been produced in dogs with neutral or alkaline diets and large doses of vitamin D. The calcification of the soft tissues produced by vitamin D in human subjects has not yielded enough anatomic data to be too significant, but the calcium deposits observed in the kidneys of all 9 patients whose data have been reviewed are probably important. The mechanisms operative in the calcific process could include all those outlined for bone disease, chronic renal disease and primary neoplasm of the parathyroid glands except increased local acidity of the bones. The fact that tissue calcification caused through the action of vitamin D is enhanced by an alkaline diet,[140] by intravenously injected sodium bicarbonate solution [187] or by a high phosphorus diet [188] should be emphasized.

The experimental use of acid and alkaline diets has been successful [173] in causing metastatic calcification in animals, especially in the case of acid or of alternating acid and alkaline diets. Mineral dietary factors may be significant in the same process in human beings, since calcium

183. Baumgartner, L.; King, E. J., and Page, I. H.: Biochem. Ztschr. **213**:170, 1929.

184. Page, I. H.: Biochem. Ztschr. **223**:222, 1930.

185. Taylor, N. B.; Weld, C. B.; Branion, H. D., and Kay, H. D.: Canad. M. A. J. **25**:20, 1931.

186. Mulligan, R. M., and Stricker, F. L.: Unpublished data.

187. Hess, A. F.; Benjamin, H. R., and Gross, J.: J. Biol. Chem. **94**:1, 1931.

188. Shelling, D. H.: Proc. Soc. Exper. Biol. & Med. **28**:298, 1930.

salts are more easily absorbed from the intestine and mobilized from the bones when the diet is acid and are absorbed in decreased amounts from the intestine and fixed in the osseous system when the diet is alkaline. However, there is no record of metastatic calcification due to a mineral diet alone having been observed in man.

Combinations of various etiologic factors may be responsible for metastatic calcification. This has been demonstrated in cases of bone disease with chronic renal disease [189] or with hypervitaminosis D,[28] in cases of chronic renal disease with hypervitaminosis D [69] with [53] or without [190] an alkaline diet and in cases of primary hyperparathyroidism with hypervitaminosis D [98] with [80] or without [89] an alkaline diet.

CONCLUSION

In human pathology, bone disease, chronic renal disease, primary parathyroid neoplasm and hypervitaminosis D, alone or in combination, have been incriminated as causing metastatic calcification. In animals, parathyroid extracts, vitamin D and mineral diets have been employed to produce metastatic calcification. Concerned in the production of this calcification are the direct effects of destructive lesions on bone, the retention of phosphate in the blood following damage of the renal parenchyma, the demineralization of the skeleton caused by the parathyroid hormone in secondary and primary hyperparathyroidism, the osteoclasis produced by hypervitaminosis D, the chemical composition of bone, the manner in which calcium salts are released from the bones into the blood, the blood transport of calcium salts whereby they reach the soft tissues and the mechanism whereby calcium salts are deposited in those tissues susceptible to metastatic calcification. In the release of the calcium salts of the bones, hyperemia, increased concentration of protein, altered concentration of hydrogen ions and phosphatase activity must be considered. In the mechanism by which calcium salts are deposited in the soft tissues, supersaturation of the serum with calcium and phosphate ions, reduction of the tissue concentration of hydrogen ions, phosphatase activity, and tissue injury due to the initial calcific changes are factors to be borne in mind. Equation reactions have been suggested by which the demineralization of bones and the calcification of the soft tissues may occur.

---

189. Virchow.[1] Askanazy.[2] Küttner.[5] Czech.[6] Plaue.[8] Heller.[8] Kockel.[9] Bender.[11] Scheele and Herxheimer.[13] Jadassohn.[17] Pari.[18] Barr.[24]

190. Lightwood.[41] Pollack and Siegal.[47] Brown and Ginsberg.[51]

Obituaries

HAROLD E. ROBERTSON, M.D.

1878—1946

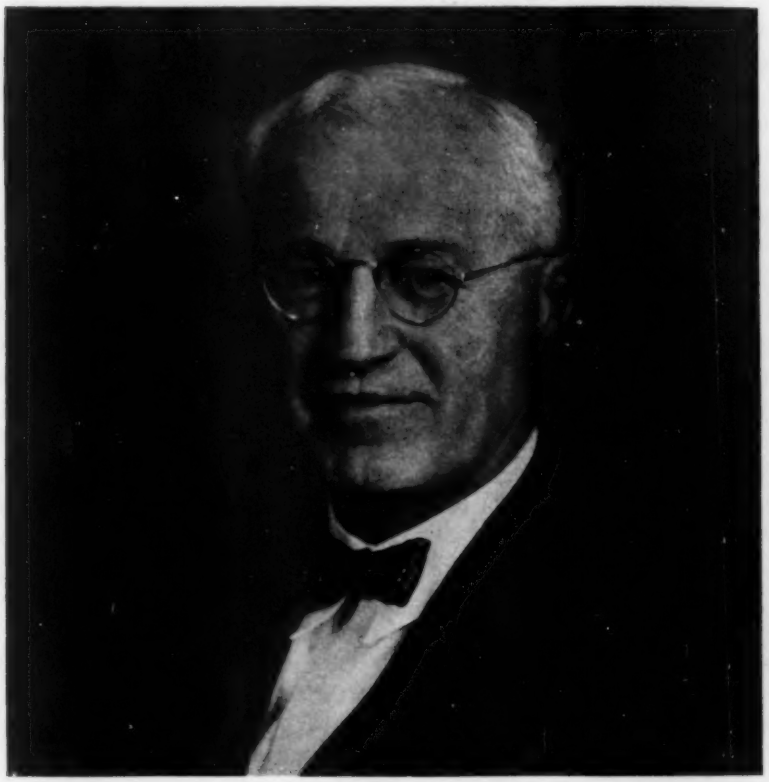
Harold E. Robertson was born Oct. 8, 1878 at Waseca, Minn. He received the degree of Bachelor of Arts in 1899 from Carleton College, Northfield, Minn., and later attended the University of Pennsylvania, from which he obtained the degree of Doctor of Medicine in 1905. After one year as instructor in pathology at Albany Medical College, he felt the need of more training and became assistant pathologist at the Boston City Hospital under the late Dr. F. B. Mallory, for whom he had great respect and lasting admiration. During this period he was an instructor in pathology at Harvard Medical School. In 1907 he returned to his native Minnesota as instructor in pathology and bacteriology at the University of Minnesota, where he became head of the department and full professor of pathology and bacteriology in 1914, which position he retained until 1921. In 1914 and 1915 he studied in Germany under Dr. Ludwig Pick and, following this, under the late Dr. Ludwig Aschoff, with whom he worked on the problem of tetanus. From 1917 to 1919 he was a major in the Medical Corps of the American Expeditionary Force, being stationed at Army Laboratory I for six months, and later at Dijon and at Paris, France. On July 1, 1921 he went to the Mayo Clinic as head of the section on pathologic anatomy, at which time he was also appointed professor of pathology in the Mayo Foundation, Graduate School, University of Minnesota. The latter position he retained at the time of his death.

Dr. Robertson had wide interest in the field of pathologic anatomy and wrote on many subjects, but he was especially interested in pulmonary tuberculosis, ulcers of the duodenum, diseases of the gallbladder and diseases of the gastric mucosa. In 1944 was published his book entitled "Hydronephrosis and Pyelitis (Pyelonephritis) of Pregnancy, Etiology and Pathogenesis, an Historical Review."

As a result of early training under the late Dr. F. B. Mallory, Dr. Robertson retained his interest in histologic technical methods. He was, in his earlier years in Rochester, much interested in the teaching museum and in museum technics, some of which he developed himself. This required much time and, as he felt he was not giving sufficient time to teaching, he helped form a separate department, the medical museum. The weekly clinicopathologic meeting which Dr. Robertson developed

in Rochester was for twenty-three years the outstanding meeting of the week and has been the model for many other conferences throughout the country.

In spite of all these activities, to those of us who remain to carry on, his greatest contribution to medicine and to the Mayo Clinic was his outstanding ability as a teacher. He was a naturally forceful speaker,



H. E. Robertson

HAROLD E. ROBERTSON, M.D.
1878—1946

and to all who listened to him he imparted his own enthusiasm. His strong personality, combined with wide and exact knowledge, gained for him a position of preeminence among his colleagues in the field of pathology. He died in Rochester, Minn., on March 8, 1946 at the age of 67 years.

Notes and News

Appointments, Etc.—In the University of North Carolina School of Medicine, Chapel Hill, K. M. Brinkhaus has been appointed professor and head of the department of pathology; F. L. Rights, assistant professor of bacteriology, and J. B. Graham, instructor in pathology.

N. Goormaghtigh, professor of pathology, University of Ghent, Belgium, will spend next April and May in the United States, under the auspices of the Belgian-American Educational Foundation.

New Cancer Journal.—The British Empire Cancer Campaign announces the publication of the *British Journal of Cancer* as its official organ. The annual subscription is $8.40; the publisher is H. K. Lewis & Co., 136 Gower Street, London, W. C. 1.

Death.—George T. Caldwell, professor of pathology, Southwestern Medical College, Dallas, Texas, died of coronary occlusion Jan. 20, 1947, aged 64. He received his M.A. degree in chemistry at Ohio State University in 1913. In 1919 he graduated in medicine at Rush Medical College, and in the same year he obtained the Ph.D. degree in pathology at the University of Chicago, where he came under the influence of H. Gideon Wells, whose methods of teaching he followed closely. From the fall of 1919 to 1943 he was professor and head of the department of pathology in Baylor University School of Medicine, Dallas, and since 1943 he occupied the same position in the Southwestern Medical College, in the same city. His wife, the former Janet Anderson, M.D., Baylor University, 1921, and pathologist in Dallas, survives him, as does the daughter, who also is a physician. Dr. Caldwell was an effective and successful teacher, administrator, organizer of hospital laboratories, tissue diagnostician, consultant and, altogether, a highly important factor in the progress of medical education and practice in his part of the country.

GEORGE T. CALDWELL
1882-1947

Society News.—The College of American Pathologists was organized in Chicago, Dec. 12 and 13, 1946. Frank W. Hartman, Detroit, is the president. The main purpose of the college is "to elevate the standards of the practice of pathology." The college is an outgrowth of the American Society of Clinical Pathologists.

The American Association for Cancer Research will hold its thirty-eighth annual meeting in the Hotel Stevens, Chicago, May 16 and 17 next.

The Society of American Bacteriologists will meet in Philadelphia May 12 to 16 next, with headquarters at the Bellevue-Stratford Hotel.

Army Medical Library Microfilm Service.—This service is now generally available for civilian physicians, institutions and research workers on a cost basis. This means direct access to the library's enormous resources of medical literature. A fee of 50 cents is charged for filming any periodical article in a single volume, regardless of length. Microfilming from monographs is furnished at 50 cents per 50 pages or fraction thereof. Photostats are also available at a charge of 50 cents per 10 pages or fraction thereof. Material filmed may not be reproduced without the permission of the copyright owner. For convenience and to keep bookkeeping costs down, a coupon system has been established. Users may buy any quantity of photoduplication coupons at 50 cents each. Order blanks are available on request. Checks should be made payable to the Treasurer of the United States, and sent to the Army Medical Library, Seventh Street and Independence Avenue, S. W., Washington 25, D. C.

Books Received

CARBOHYDRATE METABOLISM: CORRELATION OF PHYSIOLOGICAL, BIOCHEMICAL AND CLINICAL ASPECTS. By Samuel Soskin, M.D., director of the Michael Reese Hospital Research Institute; medical director, Michael Reese Hospital, Chicago, and professorial lecturer in physiology, University of Chicago; and Rachmiel Levine, M.D., director of metabolic and endocrine research, Michael Reese Hospital, Chicago. Pp. 315, with 75 illustrations. Price $6. Chicago: University of Chicago Press, 1946.

The authors, who are well known for their researches on carbohydrate metabolism, present in this book an excellent review of the state of knowledge and understanding in this field. The text consists of five parts, with topics as follows: the biochemistry and energetics of carbohydrate metabolism; introductory physiologic considerations; a critical survey of the classic criteria of diabetes; the role of the endocrine glands in carbohydrate metabolism; the integration of physiologic and clinical aspects. At the end of each chapter is a comprehensive bibliography. The book is clearly written and well illustrated. The chapter on insulin answers well the question so often asked of the physician about the mode of action of this substance. The roles played in carbohydrate metabolism by the adrenal cortex, the thyroid gland and the anterior lobe of the pituitary gland are also fully explained. The book will be of value to all who are interested in the scientific as well as practical aspects of carbohydrate metabolism.

TUBERCULOSIS IN THE UNITED STATES. GRAPHIC PRESENTATION. VOLUME 4: MORTALITY STATISTICS FOR URBAN PLACES AND RURAL AREAS IN EACH COUNTY. 1939-1941. Prepared by the staff of the Field Studies Section of the Tuberculosis Control Section of the Tuberculosis Control Division, United States Public Health Service, under the direction of Carroll E. Palmer, M.D. Pp. 190. New York: National Tuberculosis Association, 1946.

THE FIRST HUNDRED YEARS OF THE SMITHSONIAN INSTITUTION, 1846-1946. By Webster P. True, chief of the editorial division of the Smithsonian Institution. Pp. 64, with 41 illustrations. Washington, D. C.: Smithsonian Institution, 1946.

CARNEGIE CORPORATION OF NEW YORK. REPORTS OF OFFICERS FOR THE FISCAL YEAR ENDED SEPT. 30, 1946. Pp. 90. New York: Carnegie Corporation of New York, 1946.